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THE CONTROL OF THE GREENHOUSE WHITE FLY (*ASTEROCHITON VAPORARIORUM*) WITH NOTES ON ITS BIOLOGY[1].

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(With 5 Text-figures, 2 Diagrams and Plates I and II.)

CONTENTS.

1. INTRODUCTION.

THE classification of the Aleyrodidae has been recently revised by Quaintance and Baker(1) who have referred *Aleyrodes vaporariorum* Westw. to the genus *Asterochiton* Maskell. The insect, popularly known as the Greenhouse White Fly, or Snow Fly, is thought to be a native of Brazil, but is now widely distributed. Its fecundity and polyphagous habit have rendered it one of the worst greenhouse pests and it is responsible for the loss of large sums every year in the British Isles.

2. ACCLIMATISATION.

In England it breeds freely in the summer out of doors on a wide variety of plants, shrubs and trees in the neighbourhood of infested greenhouses. The vast swarms found around these in the summer and

[1] A grant in aid of publication has been received for this communication.

autumn owe their origin mainly to the adults which are continually passing out of the greenhouses and, to a less extent, to those which have been fostered in cold frames over the winter. At the same time it is highly probable that the insect can survive mild winters out of doors in the south of England and it can certainly do so in the Channel Islands where it is a more serious pest than in the Lea Valley. In the winter of 1919–20, when the insect was being bred for the purposes of this study, the adults could be found around the experimental house throughout, especially on *Althaea rosea* and *Aquilegia*. The first emergence of the

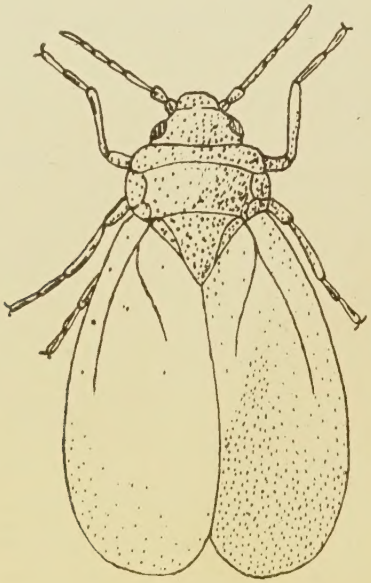

Fig. 1. Adult *A. vaporariorum* (× 100).

adults of the insects breeding outside was observed on July 1. Through the summer and autumn they were excessively numerous in the garden and became a serious pest on runner beans. At the beginning of December the greenhouses and cold frames were free from the pest but the adults were still to be found on *Digitalis* and *Althaea* where a batch of recently laid eggs was seen. Unfortunately the leaf which held these perished. In early December there was a week of snow with 23 degrees of frost on one occasion. Two adults were seen alive after this. The latter half of the month was cold and wet and no more of the insects were found in

the garden till the end of May when a few adults were seen on *Althaea* and *Philadelphus*. The Station houses were still quite free from the pest and the nearest infested greenhouse was 200 yards away from the garden. These were probably, though not certainly, survivors from the heavy infestation of the previous year.

Experiments carried out in the winter 1919–20 afford evidence that the insect may survive a mild winter out of doors in small numbers in the Lea Valley.

An *Urtica dioica*, heavily infested with all stages of the pest, was enclosed in a capacious muslin sleeve and placed outside on January 15. The adults became gradually reduced in numbers until on April 13 only 12 survived and the young foliage held numerous eggs. All the scales on the plant were dead.

A second nettle plant, heavily infested with all stages, was cleared of adults, enclosed in muslin, and placed outside on February 2. Seven adults emerged between February 13–20, and eight more between March 9 and April 13. On the latter date oviposition was occurring, but there were no intermediate stages between egg and adult. On May 20 the plant held a few adults, eggs and first stage larvae only.

A *Lamium* was subjected to a massive infestation of adults on January 14; the following day, when it was well covered with eggs, the flies were cleared from it and it was sleeved and placed outside. The eggs commenced to hatch after 87 days on April 10 and continued to do so until May 10, 117 days after oviposition.

Adults were placed in muslin-covered glass vessels containing moist soil with and without cut foliage and placed in the shade outside in January. It was found that where no foliage was enclosed they died in less than a month, January 15 to February 9 (25 days) being the longest period that one survived. In one case a *Lamium* leaf was enclosed and this kept curiously green and fresh for nearly three months. Seventeen adults were placed with it on January 15 and lived an average of 36 days, five survived 50 days, and the last one died on April 6, the 82nd day.

The outside shade temperatures during this period will be found in Table I. Although the winter was a mild one all the insects concerned in these experiments experienced frost and the adult which survived 82 days was subjected to frost on 25 nights. It is therefore clear that both the eggs and the adults are able to withstand considerable cold, but that the intermediate stages are less resistant. Both the resistant stages are dependent on living foliage; as the adult, when subjected to the alternate cold of night and warmth of day, requires food, and the

eggs on foliage severed from the plant shrivel and die. Even were this not the case the larva has not that power of movement enabling it to pass on to living plants.

In *Al. citri* the wintering stage is the pupa (2) and the phenomenon of partial brooding, such as is familiar in the case of many Lepidoptera, occurs, some of the pupae going into the wintering condition quite early in the season and the proportion which so delay emergence increasing as the cold weather approaches. No such partial brooding occurs in *Ast. vaporariorum* and its dependence on living foliage shows that it is not fully adapted to a temperate climate where the occasional occurrence of severe winters, when all foliage except that of leathery evergreens is cut down, must exterminate it out of doors.

Table I. *Showing shade temperatures (degrees F.) in greenhouse and outside during investigation.*

	Outside		Greenhouse	
	Mean	Range	Mean	Range
1919				
December	41·2	24–53	60·6	48– 76
1920				
January	39·2	21–54	62·9	50– 87
February	41·4	23–54	63·2	45– 83
March	46·0	26–73	66·3	43–100
April	50·5	29–71	66·8	44–101
May	55·2	27–86	74·7	51–101
June	59·9	33–87	71·5	47– 96
July	60·0	41–84	71·1	53– 92
August	58·2	41–83	70·4	49–100
September	57·7	36–80	—	—
October	49·0	23–76	—	—
November	42·2	20–58	—	—
December	39·9	9–53	—	—

3. FOOD PLANTS.

The insect has a wide range of food plants but those which suit it best have rather thick sappy leaves and among its most favoured hosts may be mentioned the following: tomato, potato, cucumber, vegetable marrow, French beans, tobacco, hollyhock, calceolaria, dahlia, heliotrope, stinging nettle. On these plants practically every egg laid produces an adult under favourable circumstances. On a number of hard leaved plants it can breed successfully but the mortality of the larvae is great and the plants do not frequently become massively infested. Such plants are the grape vine, various fuchsias, *Calla*, begonias, geraniums.

On the younger foliage of the tuberous begonias none of the scales survived the first moult and on the older leaves scales at the extreme periphery alone reached maturity. A similar thing occurred on fuchsias of the Mrs Marshall type where frequently a leaf was found with a complete fringe of mature or empty scales while on the rest of the leaf all the scales were dead. On chrysanthemums breeding was free on old foliage but not common on young growth. On two weeds strongly favoured by the adults, *Solanum dulcamara* and *Lamium purpureum*, no scale was ever found to mature, all dying either before or just after the first moult. On narcissus, tulip, hyacinth and various grasses eggs were often laid, but no larvae passed the first moult. Mature scales have been found rarely on elder and hawthorn and rather frequently on elm. This list by no means exhausts the food plants of the insect which were noted, but is merely indicative of its range.

4. HABITS OF ADULTS.

The adults usually mate on the leaf on which they emerge and frequently commence oviposition there. Later they seek younger foliage. Outside, when there is a perceptible wind, they are very reluctant to take flight, but on warm still days they may sometimes be seen hovering in numbers over their host plant. In the tomato houses they often remain very localised until the infestation on a few plants has become massive. Trimming the plants and the consequent disturbance aids their dispersal. They are distinctly gregarious as the following figures show, the counts being made in each case on foliage on which no adults had emerged.

On July 8, large bushy *S. dulcamara* growing under staging in the greenhouse, 260 leaflets all young and tender, plant held 90 flies distributed on 35 leaflets and of these 15 (16·6 per cent.) were on one leaflet and 9 (10 per cent.) on another.

On July 16, 10 plants, *Trifolium sativum*, growing in a box in the greenhouse held 242 flies distributed as follows: 7, 4, 35, 79 (33 per cent.), 16, 11, 3, 0, 53, 34.

On July 20 the top 40 leaves of an *Al. rosea* held 129 flies of which 55 (43 per cent.) were on the 22nd leaf from the top.

On August 27, a young *Urtica* held 32 flies of which 22 (68 per cent.) were on one leaf and the others distributed over the remaining 11 leaves.

This gregarious habit has possibly originated for the better protection of the scales from parasites. If a healthy scale is watched under a moderately high power of the microscope and in bright sunlight, the "honey dew" excreted at the anus in the base of the lingula is seen to

form into bubbles which swell up very suddenly and burst, distributing the dew as a fine spray. The mechanism of the bubble formation has not been made out. There is at the caudal end of the vasiform orifice a small trumpet-shaped organ and the tip of the lingula frequently touches the mouth of this. The trumpet-shaped organ lies at the end of the furrow running from the caudal air channel of the scale to the vasiform orifice. No actual air channel was followed, and time did not permit of any exhaustive examination of structure. A large number of scales blowing bubbles in this way would form a continuous shower of a sticky spray and it seems reasonable to suppose that this would be a deterrent to the small parasitic hymenoptera. Bubble formation is well known in another group of the Hemiptera, certain Cercopidae, the well-known "froghoppers" which form the "cuckoo spit."

The adults exhibit a remarkable colour reaction, being strongly attracted to yellow, and to green and orange in proportion to the amount of yellow these colours contain. The experimental work on this subject will be recounted elsewhere.

(1) *Length of life.* A study of the length of life of the adults, their fecundity and parthenogenesis was carried out with newly emerged females, alone or with single males, on small plants growing in muslin covered beakers. In this way daily observations could be made without disturbing the insects and these could be transferred to fresh uninfested plants before their offspring attained maturity. Unfortunately *Lamium purpureum*, a plant which thrives well under these artificial conditions, was used in a number of the earlier experiments and as none of the young matured these were largely wasted.

The average life of 16 females, including three which came by accidental deaths, was 40 days. The longest life recorded was 104 days, on *Lamium*. The average life of 10 males was 25 days, the longest being 46 days, also on *Lamium*.

(2) *Fecundity.* The average number of eggs laid was 130 per female and the rate of oviposition averaged about three eggs a day. The largest number laid was 534 giving an average of slightly more than five a day. There appeared to be some variation in fecundity in accordance with the food plant, such as Morrill and Back(2) describe for *Al. citri*, but the experiments were insufficient for this to be certain. Oviposition began on the second to fifth day after emergence in most cases. The high mortality of the young, which occurred several times on suitable foods, was due to the difficulty of keeping all the foliage healthy under conditions ensuring that no invading insects could contaminate the experiments.

Table II. *Showing the length of life, fecundity, and sex of offspring of* A. vaporariorum, *mated and unmated.*

Experiment No.	Date commenced	Plant	Condition of female	Life in days		Number of eggs	Offspring	
				Male	Female		Males	Females
5	20. I.	*Ranunculus*	Virgin	—	9+(escaped)	4	3	0
6	26. I.	*Lamium purpureum*[1]	,,	—	71	186 } 271	—	—
		Urtica dioica[2]				85 }	76	0
8	26. I.	*Lamium purpureum*[1]	,,	—	77+(escaped)	147 } 297	—	—
		Urtica dioica[2]				150 }	107	0
9	26. I.	*Lamium purpureum*[1]	,,	—	17	82	—	—
21	6. II.	Tomato	,,	—	46	110	73	—
40	12. IV.	*Urtica dioica*	,,	—	22	88	8	—
7	26. I.	*Urtica dioica*	Mated	8	55	191	33	123
10	27. I.	*Lamium purpureum*[1]	,,	46	104	534	—	—
11	27. I.	*Senecio vulgaris*	,,	7	20	12	0	1
20	6. II.	Tomato	,,	34	39	74	16	12
22	6 II.	*Trifolium pratense*	,,	45	47	83	14	13
25	18. II.	*Urtica dioica*	,,	33	33	102	11	11
26	18. II.	,,	,,	13	14	28	15	8
33	12. III.	,, (out of doors)	,,	40	95+(drowned)	95	No record	
39	12. IV.	*Solanum dulcamara*[1]	,,	5	13	49	—	—
41	20. IV.	*Urtica dioica*	,,	16+(killed)	16	63	2	8

[1] Food plant unsuited to larvae.

[2] Transferred to this plant.

(3) *Mating.* Mating occurred soon after emergence and it is the habit of the male to rest quietly by the side of the female and to effect impregnation repeatedly. In one case coitus was observed between the same pair on five occasions between January 27 and February 26 and probably also on March 6. Notes were made of their relative positions

on 37 days and on 23 of these they were close together. This repetition appears to be unnecessary as in one case when coitus occurred on January 29 and the male died on February 3 the still isolated female continued to oviposit until March 19 and the effect of the fertilisation was evident to the end in the sex of her offspring, the eggs laid from January 30 to March 2 producing 82 females and 21 males and those laid from March 2–19 producing 41 females and 12 males.

(4) *Parthenogenesis.* Morrill first noted parthenogenesis in Aleyrodidae and in conjunction with Back(2) found that unfertilised eggs of *Ast. vaporariorum*, *Al. citri* and *Al. nubifera* always gave rise to male offspring. Hargreaves(3), working in England, found just the reverse, and bred two generations of females of *Ast. vaporariorum* in the absence of males and states "out of the hundreds of flies that I examined I did not encounter a single male." Williams(4) found several colonies of the insect in England consisting entirely of females or in which this sex largely predominated. In his experiments in breeding he obtained from mated females small families in which the sexes were equal but none of the offspring of his virgin females reached maturity. This author and later Schrader(5) discuss the genetics of the insect and the suggestion is made that the parthenogenetic female producers in England may have arisen by mutation from the American parthenogenetic male producers of America and that the occurrence of some males in England may be due to fresh importations.

In the greenhouses in the Lea Valley the sexes occur in approximately equal proportions, counts giving in July out of 305 insects a male percentage of 52·8 and in October out of 118 a male percentage of 46·6. The breeding experiments show that the strain agrees with the American race in this important point, as the data in Table II show. Five virgin females produced offspring totalling 267 all of which were males. Seven mated females produced offspring also totalling 267 and of these 91 (34 per cent.) were male and 176 female.

5. DEVELOPMENT.

(1) *Egg.* The eggs (Fig. 2) (Plate I, fig. 1) are laid in circles on smooth leaves, but on hairy leaves like those of the tomato they are scattered in groups. A firm attachment to the leaf is gained by means of a short stalk which rests in a cut made by the female. Like all the other stages of the insect they are covered with wax. At first they are greenish yellow in colour but darken in two or three days in warm weather and during the greater part of the incubation period they are quite black.

They are almost invariably placed on the undersides of the leaves. As indicated above the incubation period may be very prolonged in cold weather outside. The varying incubation periods were recorded on a series of plants from December to August and the results are summarised in Table III. The longest period observed outside was 117 days and the shortest 13–16 days in August, mean temperature 58° F. The incubation period outside in August is little more than half that recorded in the greenhouse in December, though the mean temperature in the latter case was two degrees higher and is approximately the same as that in April under glass with a mean temperature of 67°. This seems to show

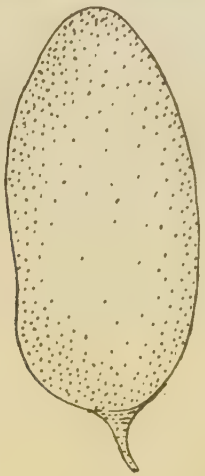

Fig. 2. Egg of *A. vaporariorum* (×350).

that sun heat is more stimulating than artificial, the sunshine hours in the three months being: December, 27; April, 87; August, 158.

(2) *Scale.* The characteristics of the four scale stages are described by Hargreaves(3). The first larva (Fig. 3*a*, *b*) moves about on the surface of the leaf but usually only a sufficient distance for it to grow without coming in contact with others from the same batch of eggs. The movement was usually confined to a few hours and once only was one seen to move the day after hatching. On one occasion a larva was seen walking on the stem of a plant, a very small *Trifolium pratense*, the leaves of which were overcrowded with scales. When cut foliage heavily infested with hatching eggs was placed on the soil around the stems of *Urtica*

and beans no migration of the larvae from the dead leaves to the living plants occurred. When eggs were hatching in numbers on the unsuitable foot plant *Lamium*, and it was particularly desired to preserve the larvae, an *Urtica* was planted by the side of the former and the leaves of the two were stitched together. No larvae passed from the unsuitable to

Table III. *Showing the duration of the egg and scale stages of* A. vaporariorum *on various plants in a heated greenhouse and outside.*

	Plant	Date of oviposition	Date of hatching	Duration of egg stage (in days)	Date of emergence of adults	Duration of scale stage (in days)	Number emerged
In heated greenhouse	Tomato	3–4. XII.	23–29. XII.	20–25	20–28. I.	28–30	Many
	Urtica	3–4. XII.	23–29. XII.	20–25	21–28. I.	29–30	,,
	Anemone (St Brigid)	14–16. I.	1–4. II.	17–21	9–18. III.	37–43	28
	Bean	28–30. I.	14–19. II.	17–20	18–31. III.	33–41	260
	Tulip	20–23. II.	11–15. III.	20–21	—	—	0[1]
	Bean	23–24. II.	13–15. III.	19–20	7–17. IV.	25–33	49
	Begonia (tuberous)	2–3. IV.	16–19. IV.	14–16	—	—	0[2]
	Urtica	2–3. IV.	16–18. IV.	14–15	12–18. V.	26–30	87
	Potato	23–25. IV.	5–8. V.	12–13	26. V.–4. VI.	21–27	270
	Tomato	12–13. V.	21–22. V.	9	8–18. VI.	18–27	870
	Bean	9–10. VII.	17–19. VII.	8–9	3. VIII.?	17–?	Many
Outside	*Lamium*	12–15. I.	10. IV.–10. V.	87–117	—	—	0[1]
	Urtica	5–8. III.	28. IV.–14. V.	54–67	—	—	0[3]
	Urtica	2–3. IV.	14. V.–20. V.	42–48	—	—	0[3]
	Urtica	5–19. IV.	20. V.–1. VI.	43–45	29. VI.	40	5
	Bean	—	8–10. VIII.	—	7. IX.–?	30–	Many
	Bean	2–3. VIII.	15–18. VIII.	13–15	15–20. IX.	30–33	Many

[1] Food unsuited to larvae.

[2] Young begonia foliage unsuited to larvae.

[3] All infested leaves shed.

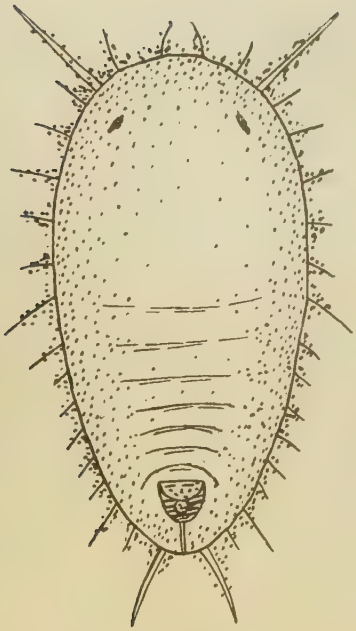

Fig. 3 *a*.

Fig. 3 *b*.

Fig. 3. First stage larva of *A. vaporariorum*, recently hatched (×300). *a*. dorsal view, *b*. lateral view.

the suitable food. The movement of the larvae is therefore not a migratory one and when the eggs are laid on an unsuitable plant or the foliage holding them is severed from the plant, they cannot survive. Eggs and feeding larvae on severed foliage shrivel up and die with the drying of the foliage which holds them.

All the larval stages are distinctly flat after the moult and the growth in each stage is in depth only. The first three become very turgid towards the moult and the skin splits at the junction of the thorax and abdomen by a T-shaped orifice. Through this the larva protrudes the head and thorax and forces itself forward till it can grasp with its legs the leaf in front of the old skin. It then elevates the posterior end of the body thus tearing the old skin away from the leaf. This is heavy through being filled with the "honey dew" and when it is released it falls clear of the leaf as a rule. Walking subsequent to a moult has not been observed but when the scales are crowded a revolving motion is often seen while the larva feels for a clear space. Overlapping of the scales, however, is common in heavy infestations. When the adult emerges from the mature scale the empty shell is left attached to the leaf.

The fourth stage of the scale (Fig. 4) is always referred to in the literature of the Aleyrodidae as the pupa, but at the beginning of the instar it is as much a larva as the preceding stages. Its dorsal skin becomes somewhat heavily chitinised and leathery and in its growth this is elevated entire from the leaf, a corrugated palisade of wax forming as the elevation proceeds. The stout waxen case is continued entirely over the ventral surface and the mouth stylets protrude through it. Respiration takes place at folds where the otherwise translucent wax remains opaque white and porous. These breathing folds are the same depth as the palisade and are situated one median posterior and two antero-lateral in the region of the thorax. The dorsal surface carries a marginal fringe of short tooth-like waxen processes arising from bosses. These short spines curl downwards. There is also a system of longer waxen processes standing upright from the scale. Hargreaves mentions and figures eleven pairs of which seven pairs are marginal. In the specimens examined during this work only four pairs could be distinguished from the marginal teeth, viz. one anterior, one over the lateral breathing folds, one posterior to this pair at the level of the first abdominal segment, and one caudal, while in the typical form those on the disc agreed with Hargreaves' description, viz. one pair cephalic, one thoracic, one on the third and one on the fourth abdominal segments. In a few of the specimens mounted from tomato the fifth and sixth abdominal segments

also bear spine bosses, and in one case there is an asymmetrical one on the fifth segment. Where these additional ones are not developed, minute hairs can be seen replacing them, evidently vestiges.

This instar becomes quite opaque early in its development and it is therefore not possible to tell by the movements of the pharyngeal pump how long it continues to feed. After the eyes and other organs are well developed, if it is removed from the leaf its mouth stylets wave to and fro as though they were still functional. Considerable growth in depth also takes place after the adult eyes are distinct. If it is removed from

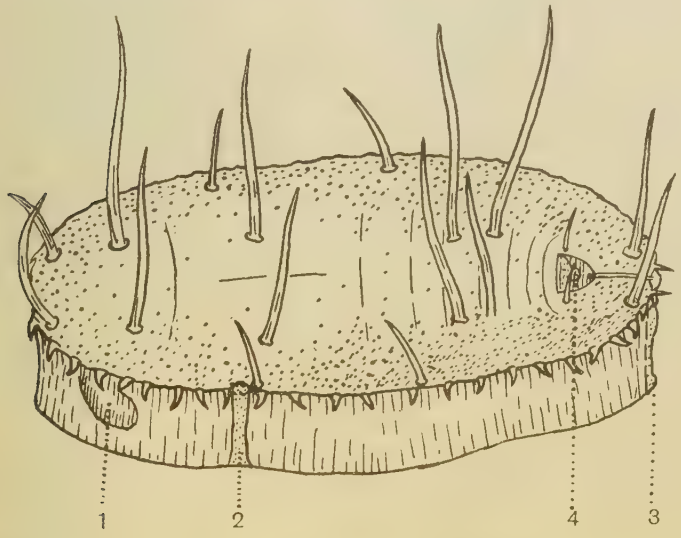

Fig. 4. Mature scale (pupa) of *A. vaporariorum* (× 130). 1. adult eye, 2. thoracic breathing fold, 3. caudal breathing fold, 4. vasiform orifice.

the leaf shortly before emergence the mouth stylets break off short by the case and have evidently ceased to function except as an anchor. About this time a copious excretion of honey dew takes place and the insect then lies tolerably free inside the old larval skin.

The duration of the scale stage was noted on a variety of plants which were examined before use to ascertain that they were free from the pest. They were then heavily infested with the fly for one or two days. After this the fly was cleared from them and they were enclosed in muslin sleeves. The hatching of the eggs and the emergence of the adults were

noted. The limits of the scale stage in the greenhouse were found to vary from 45 days in February to 17 days in July and bore a close correspondence to the temperature (see Tables I and III). There was however some variation with the different species of plants, since in one experiment when a variety of plants were infested on April 23–25 and the eggs on all hatched from May 5–8 the duration of the scale stage was on fuchsia 26–31 days, on thick-leaved zonal geranium 24–30 days and on tomato, potato, calceolaria, and thin-leaved variegated geranium 21–29 days.

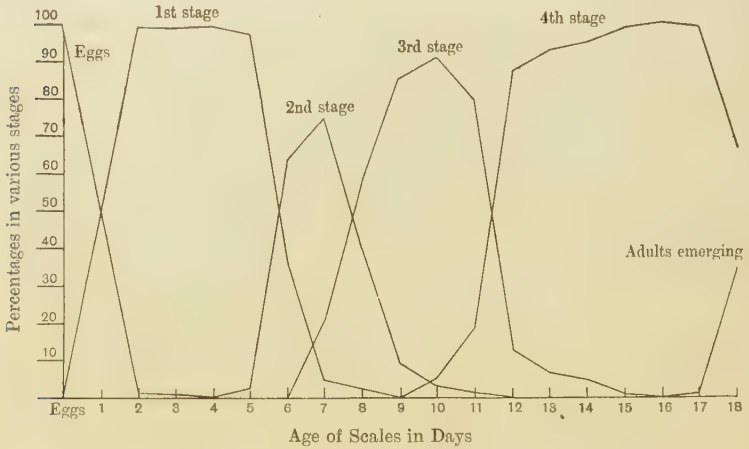

Diagram I. Showing the time spent in the four larval stages by *A. vaporariorum* on French beans at optimum temperature (mean 74° F.). The maximum number moult where the curves cross at 50 %.

In July young bean plants, known to be free of the insect, were taken one each day for 19 days and exposed for 24 hours to a large number of adults. These were then cleared off and the plants were kept in a fly-free muslin cage. When emergence of adults commenced on the plant first infested the series was stopped, each plant holding developmental stages of the same age from egg to adult. A portion of each plant holding 200–450 scales was then examined and each scale was assigned to its particular instar, the condition of 4900 scales being thus noted. The percentages in the various stages on each plant are shown in Diagram I. The mean temperature during the experiment was 74° F. with a range of 54–193°. Hatching began on the 8th day after oviposition and was practically complete by the 10th day. The figures showed that on the

average the duration of the first stage was 5 days, the second 2 days, the third 3 days, and the fourth stage 8 days. On *Ranunculus* in February and March with a mean temperature of 64° (range 45–94°) the duration in days of the four stages in four scales was: 1st, 6–7; 2nd 4–6; 3rd 8–11; 4th 16–19. On beans in March and April, with a mean temperature of 67° (range 47–101°), the average duration in days of 49 scales was: 1st, 7; 2nd, 3; 3rd, 6; 4th, 12. These instances sufficiently show the proportion of the larval life which is spent in the various instars.

The emergence of the adults appeared to be always in the early hours of daylight.

6. OCCURRENCE OF *A. SONCHI* KOTINSKY IN ENGLAND.

A second form of pupa appeared in the greenhouse where the main culture of *A. vaporariorum* was kept (Plate I, fig. 2). This scale differed from the typical form in the absence of dorsal tubercles and processes and the greater development of the marginal waxen processes which stuck out parallel with the leaf surface. It was first seen on a small *Acer* which had been brought in to test its susceptibility to the fly and had been in the greenhouse for some weeks; later, on *Brassica oleracea* which had been introduced for the same purpose. Both these plants were thought to be clean at their introduction and the typical scales never developed on them. It was also found on *Polygonum aviculare* and *Sonchus oleraceus* which had grown from seed in the chamber. Outside it was found on *Acer*, *Sonchus*, *Clarkia*, *Tropaeolum indicum* and *T. canariense*. Its incidence in numbers in the Station garden corresponded to that of the typical *A. vaporariorum*, the experimental greenhouse being the focus. The same scale was seen in Guernsey in October massively infesting *Sonchus* in the greenhouses, again in association with *A. vaporariorum*. A *Polygonum* which, after pocket-lens examination, was believed to hold the atypical form only was cleared of all adults and placed in a muslin cage with three tomato plants which were uninfested. This cage was unopened for a month during my absence from the Station, watering being done through the muslin. At the beginning of October when the cage was opened there had been time for one generation to breed through. The tomato plants were found to be massively infested with typical *A. vaporariorum* in all stages. It was then thought possible that the two scales belonged to the same species as no differences in the adults could be detected. Material from the cage and from outside was therefore submitted to Mr Laing of the British Museum who reported:

"The absence of dorsal tubercles gives the form found on *Acer*, *Tro-*

paeolum and also to a certain extent on *Polygonum aviculare*. This form may be that originally described by Barensprung as *complanatum*, but his description is totally inadequate. It almost certainly is *sonchi* of Kotinsky from Hawaii, and the absence of the two tiny caudal spines would make it *tentaculatus* Bemis, from California. On *P. aviculare* I find in my preparations both typical and atypical pupa cases, but on the tomato the typical form seems to be present alone. Is it not possible that the two forms were present at the beginning and that only the typical form developed on the tomato? The case would then be very similar to Morrill's experiment when he separated *packardi* Morrill from *vaporariorum*. In addition to the absence of the dorsal tubercles in the atypical material there are other minor differences and seeing the material without knowing anything of the experiment I should unhesitatingly have called the two forms distinct species."

It was intended to do further work with the atypical form in the present summer but circumstances have prevented this. Its wide distribution, Hawaii, Guernsey, the Lea Valley, and possibly California, and its apparent association in two of these with *A. vaporariorum* make it a very interesting form.

7. ECONOMIC IMPORTANCE.

Financial loss caused by *A. vaporariorum* results mainly through its attacks on tomatoes, beans and potatoes grown under glass. The two latter suffer less than the former because they are brief crops and the insect does not have the same opportunity of causing massive infestations on them for this reason. The damage is partly direct through loss of sap, but is mainly due to the layer of honey dew which soon covers the foliage and fruit of the infested plants. In this medium sooty moulds grow, forming a black felt over the foliage which keeps away sunlight, while all fruit from infested plants must be wiped before it can be marketed. The fungus growth on the tomato plants was examined by Dr W. F. Bewley, who found it to consist of *Penicillium* sp. predominating, together with *Cladosporium herbarum* and *Fumago vagans*, all saprophytic forms.

In order to estimate the damage done by an unchecked attack on tomatoes, 34 young plants in 12-inch pots were lightly infested with about ten adults each on May 6 and placed in a chamber in the greenhouse. On half of them the pest was allowed to develop unchecked while the others were removed about once a fortnight to another chamber and fumigated with hydrocyanic acid gas in order to keep the infestation

under control. Apart from the fumigations the plants received the same treatment. The infested plants were practically dead on August 24 and the last fruit was picked from them. Their total yield was 29 lbs. 14 oz. The fumigated plants were still vigorous at the last fruit picking on October 8 and their total yield was 66 lbs. 8 oz. or about 4 lbs. of fruit per plant. Not uncommonly tomato plants in trade nurseries are as badly attacked as these and it may be stated fairly that if the plants are infested early and the infestation is not checked a loss of more than half the crop will be the result.

Such a condition only obtains when the grower deals in mixed crops keeping his houses occupied during the winter. The grower who only grows tomatoes has his houses empty for several months and nearly always commences the season free from the pest. Invasion by the insects is liable to occur in May and June and it is not until the late summer that fumigation becomes necessary.

Although the cucumber was mentioned above among the favourite foods of the insect, trade growers of this crop do not recognise it as a pest in the Lea Valley. It is sometimes present in the houses early and late in the year, but generally disappears when the weather becomes warm. In the tomato houses it was observed that when the temperature of the air approaches 100° F. the flies become restless and flutter up to the glass, many escaping through the laps. It was found by experiments in thermostats that they are stupified when the temperature rises to 105°. Fifty flies so stupified by 40 minutes' exposure to 102–106° nearly all recovered by the second day after the experiment. One out of 20 recovered after 5 minutes' exposure to 104–110°. It was clearly impracticable to effect control of the pest in the tomato houses by so raising the temperatures so no further details of the series of experiments will be given, but they showed that it is the atmospheric conditions of the cucumber houses which often rise above 100° that prevent the white fly from becoming a serious pest there.

8. CONTROL.

The spread of the insect is greatly aided by the culpable negligence of some nurserymen who make a business of the sale of young plants. Bedding plants such as geraniums and salvias are often sold infested with the pest, and even young tomato plants are sometimes sent out in a similar unclean state. Probably only legislation could stop this dangerous practice, but growers may be advised to ask for a guarantee of cleanliness in this respect for any plants they purchase for gardens around the nurseries, and especially for young tomato plants. It is

always advisable to raise tomato plants from seed rather than to buy them from mixed growers. Propagation of the new season's crop from cuttings taken from old plants has also led to very serious infestations and though this is not a common practice it is well to warn against it.

Growers should realise that they are themselves responsible for the heavy infestations outside the nurseries, and they should prevent these by never allowing conditions inside to get bad. Very many of the insects pass out of the houses of their own accord but still larger numbers are taken out on trimmings and on the plants at the end of the season. The pest should be kept under such control that the trimmings are never heavily infested and if the plants are still infested when they are cut out at the end of the season, they should be cyanided before removal. It is a common practice to burn sulphur in the houses before the plants are removed, but this is not a good fumigant against the white fly.

The grower of mixed crops should free his nursery from the pest during the winter by fumigations of all the occupied greenhouses and cold frames. A greenhouse may be easily cleaned without fumigation by completely emptying it and digging it over to bury any infested weeds or leaf fragments holding pupae, leaving it empty with the heat on for a week to starve any adults that remain and then reoccupying it with clean plants. Much of the trouble with this pest, especially in Guernsey, is caused by propagating tomatoes in houses containing infested potatoes or beans. A small separate propagating house would prevent this early infestation of the seedlings. Attacks of white fly are often caused by sheltering some ornamental plants such as fuchsias in the greenhouses. The specialist in tomato growing should not permit this practice.

9. FUMIGATION.

Spraying is of little use against white fly, while properly applied fumigants give excellent results. Special attention was given to four fumigants, viz. naphthalene, tetrachlorethane, tobacco preparations and hydrocyanic acid gas, and these will be discussed in turn.

(1) *Naphthalene.* This substance forms the basis of a number of proprietary articles sold as exterminators of white fly and attention was given to it for this reason. Naphthalene is sold in various forms as "pure flake" which is a sublimed form; "crude naphthalene," a dark material containing carbon as an impurity and from which most of the tarry acids have been removed; "drained salts" or "whizzed naphthalene," a damp oily product containing a very variable amount of the tarry acids; "undrained salts" or "unwhizzed naphthalene" which

contains relatively larger quantities of phenols. The effect of these varies greatly, but in the proportions in which it is safe to use them they are toxic only to the adults and this makes them unsatisfactory and excessively costly owing to the necessity for repeated applications.

From 50 to 200 adult white flies on foliage were placed in half-gallon glass-stoppered jars and small quantities of pure naphthalene, cresylic acid and phenol, alone and in various combinations, mixed with ash were introduced on watch glasses. Notes were made of the rate at which the insects were stupified and after an hour the fumigants were removed and the jars were left with muslin covers. A count of the mortality was made the day after the fumigation. The results of a small series of fumigations are given in Table IV. It will be seen that the mortality was slight where naphthalene alone was used, heavy with cresylic acid or phenol, but almost total in most cases (10 out of 12) when naphthalene was used in combination with the tarry acids. The rate of stupifaction was in the same proportion as the final mortality, being slow when naphthalene alone was used. A similar series was carried out with various grades of naphthalene, 0·25 grm. in 0·75 grm. of ash being used and the fumigation lasting 70 minutes at a temperature of 66° F. The mortality was as follows:

1.	Naphthalene, pure flake	killed	4/65 = 6·1 %
2.	„ high grade white (Crow & Co.)	„	1/57 = 1·7
3.	„ crude (carbon impurities)	„	1/35 = 2·9
4.	„ „ (another sample)	„	2/60 = 3·3
5.	„ drained salts (Crow & Co.)	„	50/62 = 80·7
6.	1 grm. containing 75 % destructor refuse and 25 % volatilisable naphthalene containing some tarry acids (proprietary remedy)	„	15/89 = 16·8

Table IV. *Showing the percentage mortality of adult* A. vaporariorum *obtained* in vitro *with pure naphthalene alone and with tarry acids, fumigations lasting one hour. Temperature* 69–72° F.

	Naphthalene grms.					
	0	0·1	0·2	0·3	0·4	0·5
No tarry acids	—	—	—	—	—	3·5
	—	—	—	—	—	3·0
Cresylic acid ("pale straw") 0·1 grm.	79	—	—	—	62·6	95
	64	—	—	—	—	—
Pure phenol 0·1 grm.	66	100	99	99	98	97
	39	—	—	—	100	98
	76	—	—	—	—	—
Cresylic acid 0·1 grm. + phenol 0·1 grm.	78	—	—	85	—	100
	75	—	—	—	—	100

It is clear that the effect of naphthalene on white fly depends on the presence of the residue of tarry acids. The finding was checked by fumigations of an infested greenhouse with the various grades, but as it is at best a poor remedy these will not be detailed. The materials give an unpleasant flavour to the fruit and it must be realised that the introduction of an unknown amount of carbolic acid among growing plants is a risky proceeding. The vendors of naphthalenes state that the crude forms contain a very variable amount of tarry acids in the different samples, but they are unable to guarantee the strength. This would make it impossible to standardise a treatment.

(2) *Tetrachlorethane.* This liquid has been occasionally used for greenhouse fumigation during the last two years at the Experimental Station, and gives good results against white fly. Its use is simple as it is merely poured down the centre of the house in the evening and this is kept closed for as long as possible on the following day. As it is not a very poisonous substance it may be used in conservatories opening into dwellings where the employment of cyanide is undesirable and it has a future as a fumigant in very small greenhouses where the measurement of the small quantity of cyanide required is a difficulty. As it costs about ten times as much as cyanide fumigation the trade grower should take little interest in it.

Its action on adult *A. vaporariorum* is doubtless toxic, but it appears to kill the scales through the effect of the vapour on the wax as considerable numbers of the flies attempt to emerge subsequent to the fumigation and die when partially free from the pupa cases. It has no effect on the eggs. It has been used for a wide variety of plants including tomatoes and no damage has resulted except in one case when the foliage of three young sycamores (*Acer pseudoplanatus*) growing in pots turned brown the day after the fumigation and was subsequently shed. It may be therefore that some greenhouse plants would suffer from it. Daylight during the fumigation did not tend to damage and no preparation of the plants appeared to be necessary.

The liquid should be used at the rate of about half a pint to 1000 c.ft. of space and the fumigation should be repeated as with cyanide. The complete success of the fumigation depends on its duration, and the limiting factor to this is the weather as the house can be kept closed longer in dull than in sunny weather. A dull period should therefore be chosen when possible. The mortalities obtained in four experiments, with varying amounts of tetrachlorethane and varying durations, when uninfested plants were sleeved after the fumigations and kept under observation for two to three weeks, are shown in Table V.

Table V. *Showing the mortality of* A. vaporariorum *obtained with tetrachlorethane.*

Capacity of greenhouse	Amount per 1000 c.ft.	Duration	Temperature	Mortality	
				Adults	Scales
2500 c.ft.	1 pint	15 hours	61–85° F.	100%	($\frac{344}{350}$) 98%
4500 „	$\frac{2}{3}$ „	12 „	65–70	100	($\frac{833}{860}$) 83
2500 „	$\frac{1}{2}$ „	18 „	62–65	($\frac{97}{100}$) 97	($\frac{97}{100}$) 97
2500 „	$\frac{1}{2}$ „	40 „	51–68	100	100

(3) *Tobacco preparations.* It was recently stated by a correspondent in a trade journal that some twenty years ago when white fly first started to give trouble to nurserymen in this country it was only necessary to burn a little tobacco in the greenhouse in order to control it, but that now such preparations only stupify it. Whatever they were once, they are at any rate at present of very little use against the pest as they drive enormous numbers of the insects outside and many of those which fall are merely stupified. They have no appreciable effect on the young stages. The various tests made will not be discussed in detail. They included the burning of "shreds" at five times the strength recommended by the maker, fumigations with a proprietary nicotine and camphor fumigant, also with pure nicotine at the rate of $\frac{1}{8}$ oz. per 1000 c.ft. and $\frac{3}{8}$ oz. with $\frac{3}{8}$ oz. camphor per 1000 c.ft. In each case less than a 50 per cent. mortality of the adults was obtained. These preparations have also become almost prohibitory in cost to the commercial grower.

(4) *Cyaniding.* Fumigation with hydrocyanic acid gas or, as it is generally called, "cyaniding" is the most effective method of controlling the pest and is the only one known sufficiently economical for use in trade nurseries. High grade sodium cyanide, 98 per cent. purity (often described as 130 per cent. in reference to the strength of pure potassium cyanide), is employed, and the gas is generated by means of sulphuric acid. Potassium cyanide and phosphoric acid are sometimes used as alternatives, but they have no advantages and are more costly. The proportions in which to employ the materials are: 1 oz. of sodium cyanide in $1\frac{1}{2}$ fluid ozs. of sulphuric acid diluted with 3 fluid ozs. of water. An error which had become almost universal among Lea Valley growers and in other parts has led to most of the failures in the use of these materials in the past. Fear of the poisonous nature of the gas has led the users to drop the cyanide into the acid enclosed in a sealed envelope or some other type of paper packet. When the acid enters the packet and evolution of the gas commences the pressure prevents the free access of the acid and the heat of the reaction chars the remaining cyanide.

Later, when the packet breaks down, the acid will no longer act on the charred mass. The cyanide should be dropped free into the acid so that the reaction may be brisk and unimpeded. A so-called "Safety Cyanide Package" is on the market and this consists of a metal container the side of which is made of thin zinc foil. An additional amount of sulphuric acid is used and the zinc dissolves away completely so that the acid has free access to the cyanide. There is no scientific objection to this container. There is, however, ample time for the operator to drop loose cyanide into the acid and to move on to the next jar at a slow walking pace without detecting the slightest odour from the gas.

A series of eighty fumigations with this gas was carried out in an experimental greenhouse 18 ft. long by 20 ft. wide, height 11½ ft. to the ridge and 4 ft. to the gutter, capacity 2500 c.ft. The recommendations finally made were checked in blocks of tomato houses in trade nurseries and in greenhouses of mixed plants. Temperature, humidity, duration, time of day, size of dose, and the condition of the plants were all varied. Tomato plants in pots were always included, and some of these were of large growth growing in 12-inch pots, to study the effect of the gas on normal plants. Others, tomatoes or beans, were heavily infested with white fly in all stages and were enclosed in muslin sleeves at the end of the fumigation and were examined every day or two for two or three weeks in order to estimate the effect of the gas on the insect. In general three infested plants were included and placed in different positions and at different heights, but it was found that position made no appreciable difference. A fumigation chamber with a capacity of 350 c.ft. was also constructed, but it was found that results obtained in a chamber are useless when applied to a greenhouse owing to the difference in leakage and its use was abandoned except for a few special experiments.

Temperature. The results confirmed the work of other investigators that a somewhat low temperature (below 60° F.) renders the plants less liable to damage, but in practice as the fumigation of tomato houses is most often done in summer and early autumn the operation has generally to be carried out at a somewhat higher temperature than this. On 30 evenings from May to July fumigations were commenced at dusk and on only two occasions was the temperature of the house below 60° though artificial heat was cut off in nearly every case, and on 11 occasions it was above 65°. As will be shown presently it is possible to counteract the harmful tendency of high temperature by withholding water from the plants. It is exceedingly difficult to make any definite recommenda-

tion about temperature as, in the case of tomato plants, very severe damage with the same amount of cyanide has occurred at a temperature of 57° with unprepared plants and none at all at 69° with prepared plants of a similar soft growth. The best advice that can be given to the grower is that he should have his houses cool for the fumigation and carefully prepare the plants beforehand as described below.

The toxicity of the gas for the insect was not found to vary within the mean temperature limits of 47° and 66°, total mortalities being obtained with both at the correct dosage.

Time of day and duration. Every writer on the fumigation of greenhouses with this gas mentions the importance of not commencing the operation till dusk, but growers in many cases persist in starting several hours before sunset. Damage which means practically the death of the plant results. All tissue on which the sunlight falls is seared as though with flame, the growing points are killed and the buds and flowers cut off. The materials are always blamed for this and the operation discredited. Moreover the gas is less toxic in sunlight as the following cases show. A fumigation with $\frac{1}{8}$ oz. cyanide per 1000 c.ft. commenced four hours before sunset and lasted $13\frac{1}{2}$ hours gave a mortality of less than 50 per cent. for adults and negligible for the scales, as contrasted with a usual mortality of 90 per cent. for adults and 75 per cent. for scales in fumigations with this quantity commenced at dusk and lasting 8–11 hours. A fumigation with $\frac{1}{4}$ oz. cyanide per 1000 c.ft. commenced three hours before dusk and lasted 12 hours gave an almost total mortality for adults and 70 per cent. for scales, as contrasted with a usual total mortality for adults and total, or almost total, mortality for scales with the same quantity commenced at dusk and lasting 8–11 hours.

It has generally been the custom to recommend relatively large doses of cyanide with short exposures rather than small doses with long exposures. Sasscer and Borden (6) using $\frac{1}{2}$–$\frac{3}{4}$ oz. cyanide per 1000 c.ft., exposure one hour, obtained with this pest a mortality which was total except for eggs and late pupae. In the course of this work $\frac{1}{2}$ oz. cyanide, 1000 c.ft., exposure one hour, gave 95 per cent. mortality for adults, had practically no effect on late pupae, and destroyed about half the younger scales. The same amount, $1\frac{1}{2}$ hours' exposure, gave 100 per cent. mortality for adults and about 90 per cent. for scales. $\frac{1}{3}$ oz. cyanide, 1000 c.ft., exposure three hours, gave 100 per cent. mortality for adults and a poor result for scales. In the two last cases tomato plants were damaged, the temperature being 57° and 62° respectively. To control the infestation by these means at least three fumigations would be necessary as against

two when the smaller doses with long exposures are used. The former at the present price of materials would cost about £12 per acre and the latter about £4. Provided that the houses are opened up at dawn there appears to be no more risk to the plants with the long exposure and small dose than with the short exposure and large dose, and the former should always be employed.

Toxicity of the gas. The following account of the toxicity of the gas for the insect deals only with exposures of 8–11 hours' duration.

The eggs are unaffected and the adults are rather more susceptible than the scales. A large proportion of the adults fall to the ground directly the gas reaches them and unless there is a sufficient concentration many of these recover during the following day or two and regain the plants. Whenever the mortality of the adult was total that of the scales was always more than 90 per cent., generally more than 95 per cent. and often 100 per cent., so that if all the flies are killed the effect of the fumigation is known at once to be good, if not perfect. The results obtained on heavily infested plants sleeved after the fumigations will be given briefly under the various dosages. The study of the life-history was carried on at the same time and showed that the emergences recorded were after intervals too brief for them to have been in the egg condition at the temperatures ruling at the season when the particular fumigation was done.

$\frac{1}{2}$ oz. cyanide per 1000 c.ft.; 4 tests; mortality for adults always total; mortality for scales in three cases total; small numbers of adults emerged on one plant beginning on the 18th day after fumigation.

$\frac{1}{3}$ oz. cyanide per 1000 c.ft.; 6 tests; mortality for adults always total; mortality for scales in three cases total and in three cases very small numbers emerged beginning on the 13th, 16th, and 17th days respectively.

$\frac{1}{4}$ oz. cyanide per 1000 c.ft.; 14 tests; mortality for adults always total except in one case when three out of 500 recovered; mortality for scales in seven cases total; in three cases a single scale survived out of hundreds, the flies emerging on the 8th, 14th and 15th days respectively; in four cases emergence occurred in very small numbers (one to three a day) commencing once on the 10th, twice on the 14th, and once on the 17th days respectively.

$\frac{1}{5}$ oz. cyanide per 1000 c.ft.; 10 tests; mortality for adults always total except in two cases, 1 and 9 surviving respectively, in each case out of many hundreds; mortality for scales in six cases total; in two cases emergence occurred in small numbers after the 12th day, in one case two flies emerged on the 5th and no more to the 10th day; the remaining case was an unexplained failure, only 75 per cent. of the scales being killed.

$\frac{1}{6}$ oz. cyanide per 1000 c.ft.; 9 tests; mortality for adults total in five cases, and in the remainder over 95 per cent.; mortality for scales total in three cases; emergence in the other cases in very small numbers began on the 2nd, 5th (twice), 6th, 7th and

10th days respectively. In one case where an exact count was made after a fumigation lasting 9 hours on six tomato plants holding scales from 9 days old to mature pupae the mortality was 91 per cent. (2900 out of 3184).

The fact that the small numbers surviving these fumigations were apparently young for the most part at the time of treatment is difficult to explain as, when smaller doses of cyanide were used, it was found, as other workers have stated, that the pupae, which would give rise to adults in two or three days' time, were the most resistant forms. Series of plants were prepared as described above so that in each series one plant held scales representing one day's development from egg to adult;

Diagram II. Showing percentage mortalities of scales of *A. vaporariorum* of various ages obtained with small doses of cyanide, long exposures. Compare with Diagram I. The gaps on the lower curve are due to the death of three plants. $\frac{1}{8}$ oz. cyanide per 1000 c.ft. $\frac{1}{7}$ oz. cyanide per 1000 c.ft.

i.e. at one end of the series was a plant holding only eggs, due to begin hatching, and at the other end was one holding mature scales from which emergence of flies had just commenced. Two series were cyanided as follows: (1) 18 tomato plants representing complete development except that three plants died from stem rot and caused gaps; fumigated with $\frac{1}{8}$ oz. cyanide per 1000 c.ft., 9 hours' duration; (2) 19 bean plants representing complete development, fumigated with $\frac{1}{8}$ oz. of cyanide per 1000 c.ft.; $9\frac{1}{2}$ hours' duration. After the treatment the plants were kept for a week and a count was then made of the living or emerged scales and the dead ones, which had by this time turned brown and dried up.

The smallest count made on any one plant was 140 and the highest 970, while the average number dealt with was 460. The mortalities on the several days reduced to percentages are shown in Diagram II. The mortality on a tomato plant uncyanided but otherwise similarly treated was 29 dead out of a total of 900 scales, or 3 per cent., while the natural mortality on uncyanided beans was also negligible. If these two curves, and especially the lower one given by $\frac{1}{8}$ oz., are examined in relation to Diagram I which shows the proportion of the scales which would be in the different stages on each day, it will be seen that there are very suggestive drops in the mortality which correspond roughly with (1) the first moult on the sixth day after hatching; (2) the second moult on the eighth day, seen only in the upper curve; (3) the third moult on the tenth to twelfth days, and (4) the time of true pupation just before emergence begins. The mortality of the adults given by these small doses varied with $\frac{1}{7}$ oz. from 95–100 per cent. (average of five tests 98 per cent.), and in 10 tests with $\frac{1}{8}$ oz. from 88 per cent. to nearly 100 per cent., but was never total with the weaker charge.

After these experiments it appeared possible that a very good result could be obtained with two fumigations with a small charge on successive nights, and two heavily infested tomato plants were treated with $\frac{1}{8}$ oz. cyanide per 1000 c.ft. On the following morning one of them was removed from the fumigation greenhouse while the other was treated again the next night with the same charge. The mortality of the scales in all stages, on the first plant was 75·8 per cent. (805 out of 1045) and on the plant treated twice 91·8 per cent. (903 out of 1013). The combined effect of the two thus gave a less satisfactory result than would have been obtained with $\frac{1}{6}$ oz. in one fumigation.

The very small charges will clearly give a moderately good check to the pest, though they will not exterminate it, and it is at times advisable to use them on soft sappy tomato plants which for one reason or another cannot be brought into a proper condition to withstand the normal dosage.

Dosage. From these experiments and tests made in commercial houses it was concluded that in an isolated greenhouse in a moderate state of repair $\frac{1}{4}$ oz. of cyanide per 1000 c.ft. could be relied on to give practically total mortality for all stages except the eggs, if the fumigation lasted through the night. In a block of greenhouses in decent repair which communicate with one another a dose of this size is not required as the proportional leakage is less and $\frac{1}{5}$ oz. per 1000 c.ft. is a sufficient quantity, or when the houses are new and in very good repair even $\frac{1}{6}$ oz. gives almost total mortality.

The amount of cyanide which should be put into one jar depends on the width of the houses which vary in the Lea Valley from 12 to 30 feet. A good rule to follow is to so arrange the jars that the distance between two is approximately the width of the house as this arrangement gives an even distribution of the gas. Quarter ounce charges are recommended for houses up to 14 ft. wide, half ounces for houses 14–20 ft. wide, and ounces for wider ones. It is not wise to use a larger charge than this in tomato houses as the concentrated gas evolved so close to the plants is liable to cause damage.

The use of liquid hydrocyanic acid has recently been advised by Quayle(7) for the fumigation of fruit trees as an alternative to the jar method of generation, but the difficulties of distribution make this impracticable in large greenhouses.

Repetition of fumigation. The fumigation should be repeated when all the eggs have hatched but before any of the young can become adult. A reference to Table III shows that at any temperature there is an interval of a few days between these periods in heated greenhouses. The most suitable days for the second fumigation are shown in the poster "Cyaniding Tomato Houses."

Effect on plants. While the doses of cyanide mentioned above may be applied without hesitation to most greenhouse plants, very considerable caution is required in applying them to tomatoes, whether growing in pots or in borders, as this plant is particularly susceptible to damage by the gas. Hard growing wiry tomato plants resist the gas much better than soft sappy ones, but in trade nurseries the plants are usually of the latter type (Plate I, fig. 3).

The damage to which they are liable consists of burns on the foliage which may develop at once or several days after the fumigation. The damage is symmetrical on the leaflets and in moderate cases is confined to the basal half on each side of the midrib. Leaves which are fully grown are much less liable to damage than the younger ones. In mild cases the leaflets of the younger leaves crinkle up without any browning. On several occasions the only damage which has occurred has been a scorch on the underside of the petioles of two or three leaves which causes a permanent dwarfing of the leaf. The leaflets develop normally but become very crowded, while sometimes the leaf coils spirally round the main stem. The growing points and stems and trusses are only damaged by exaggerated carelessness such as fumigating in daylight or by the use of excessive doses. Faulty setting of the fruit could never be associated with fumigation.

By taking continuous measurements of the growth of very vigorous plants by means of a Farmer auxanometer it was found that when the fumigation commences a distinct check in growth occurs when doses of $\frac{1}{4}$–$\frac{1}{6}$ oz. per 1000 c.ft. are used, even if no subsequent lesions develop. After a day or two a normal rate of growth is resumed. One of these growth records is reproduced in Fig. 5. The plant from which this was taken was young, about $2\frac{1}{2}$ ft. in height and of a moderately soft nature, with the second truss setting. One pint of water was given daily. The thread was tied close behind the growing point and the pointer was reset at noon each day. The actual growth on six successive days was as follows, the fumigation lasting on the third period from 9.0 p.m. to

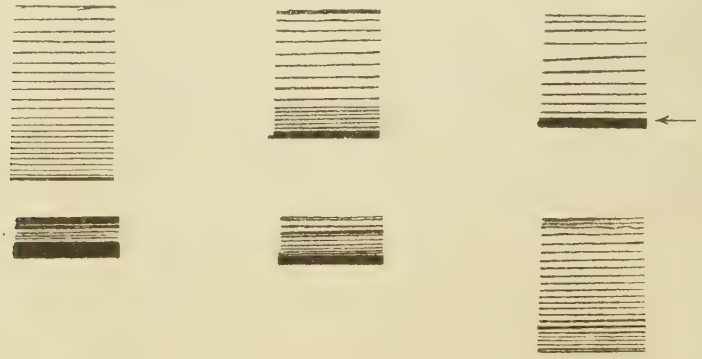

Fig. 5. Record of daily growth of tomato plant measured by Farmer auxanometer, showing check and recovery in growth after fumigation with hydrocyanic acid ($\frac{1}{4}$ oz. cyanide per 1000 c.ft., duration 9 hours). Instrument set to record double the growth. The gas was introduced on the third day at the point marked by the arrow.

6.0 a.m.: (1) 1·25 mm., (2) ·9 mm., (3) ·8 mm., (4) ·3 mm., (5) ·35 mm., (6) ·95 mm. Very slight damage to the foliage followed the fumigation. A similar check in the growth of the length of the leaves also occurs though unaccompanied by any obvious damage.

A natural assumption is that the gas causes damage by entering the leaf through the stomata but this is not necessarily the case. In this connection two experiments suggested by Prof. V. H. Blackman were carried out. Two tomato plants were placed in a dark chamber 5 hours before dusk, while four similar plants were kept in the light. At dusk all the plants were placed close together and fumigated with $\frac{1}{4}$ oz. of cyanide per 1000 c.ft., duration $9\frac{1}{2}$ hours, temperature 69–60°, relative humidity 97 per cent. The experiment was repeated with a plant kept

in the dark 6 hours before dusk and then fumigated with a control plant of similar nature; $\frac{1}{4}$ oz. cyanide per 1000 c.ft., duration $9\frac{1}{2}$ hours, temperature 61·5–53°, relative humidity 81 per cent. The plants kept in the dark would presumably have had more opportunity to close the stomata but were in no way protected against damage thereby as in each experiment precisely similar burns developed on all the plants in each test. The second experiment consisted in cutting with scissors the laminae of the leaflets parallel to the chief lateral veins, and in cutting off the tips of some of the leaflets. Four plants were treated in this way immediately before fumigation so that the gas had free access to the raw tissue. Although moderate burns developed no damage could be associated with the cuts and the injury was much the same on cut and uncut plants. After these experiments Prof. Blackman agreed that no simple explanation of the mode of injury could be given and it is not proposed to discuss it further.

It was abundantly clear, however, that after daylight the most important factor in fumigating with this gas was the turgidity of the plant. An idea prevailed among the growers, and had found expression in a tradesman's circular, that flaccid plants were very liable to damage. For this reason flaccid and very turgid plants were repeatedly fumigated side by side and in each case the foliage of the former escaped injury while that of the latter was damaged (Plate II, figs. 4 7). On four occasions series of five to eight large tomato plants in 12-inch pots were prepared by withholding water from them on successive days till one or two were flagging and one was excessively turgid while the remainder were in intermediate conditions. On two occasions the test consisted of the fumigation of two plants only, one flaccid and one turgid in each instance. The fumigation greenhouse was heavily saturated with moisture, except in one case, and the plants were then treated with $\frac{1}{4}$ oz. cyanide per 1000 c.ft., duration 9 hours. Flaccid plants and those approaching flaccidity escaped any damage under the following conditions of temperature and relative humidity: (1) 60–54°, 97 per cent.; (2) 66·5–61°, 88 per cent.; (3) 67–59°, 67 per cent.; (4) 69–60°, 97 per cent.; (5) 58–55°, 90 per cent.; (6) 65–52°, 85 per cent. In each case turgid plants side by side with the others were badly damaged, the injury being always proportional to the turgidity. The two following experiments carried out under still more adverse conditions illustrate the same point:

(1) Two plants with four trusses set, one plant turgid and one flaccid, were fumigated with the excessive dose of $\frac{1}{2}$ oz. cyanide per 1000 c.ft., duration 9 hours, temperature 64–59°, relative humidity 86 per cent. The turgid plant had its foliage

very severely burnt while that of the flaccid one was undamaged but its buds and flowers were injured.

(2) Two young plants with first truss in flower, growing in 8-inch pots, one turgid and one flaccid, were fumigated in a chamber (350 c.ft.) at the rate of $\frac{1}{4}$ oz. cyanide per 1000 c.ft., the equivalent of at least double this charge in a greenhouse; duration $2\frac{1}{2}$ hours, temperature 90–72°, relative humidity 80 per cent. Even at this excessively high temperature the flaccid plant was undamaged while the top of the turgid one was killed and the whole plant very severely scorched.

Nothing in this experimental work afforded any evidence that the humidity of the atmosphere of the greenhouse was a factor which needed to be considered in the fumigation and when plants were sprayed with water immediately before the operation no damage could ever be associated with this. Previous workers have disagreed about the importance of this factor(s) and it is probable that some have failed to dissociate the moisture of the air from that of the soil, the latter being all-important. It is fortunate for the tomato grower that air humidity is not a factor as the tomato house has necessarily a very moist atmosphere when it is closed down.

It is not, of course, practicable to allow tomato plants on which the fruit is setting to flag as the crop would be thereby damaged, but the principle has a practical application in that the grower may be advised to get the roots of the plants as dry as possible without harming them. It is a common practice in the Lea Valley to drench the borders heavily before planting (in one rather exceptional case to the equivalent of a 9-inch rainfall) so that the plants can grow for two or three months without heavy watering. (This makes the plants throw deep roots.) When the houses are in this condition there is clearly no control over the turgidity of the plants. Those who grow in pots usually keep the soil very wet while the first four trusses are setting. Under these circumstances it is advisable to use only half the quantities of cyanide recommended above which will give a very fair check to the pest and prevent it from getting out of control. Later the plants in borders are watered periodically according to the character of the soil, generally once a week, and older plants in pots do not suffer if they are allowed to dry until the "pot rings hollow." The fumigation with the full amount of cyanide may be given the night before the periodical watering is due or when the soil in the pots is dry. The day after the fumigation the plants may be watered freely.

It is not possible to give any guarantee that with these doses no damage to the foliage will occur, but an assurance may be given that

if all the rules are followed any injury will be negligible in proportion to that caused by an unchecked infestation of the pest.

(5) *Summary of Cyaniding.* A poster giving the essential instructions on the cyaniding of tomato houses has been issued from the Lea Valley Experimental Station. The foregoing instructions are summarised there and reference is made to several important points which are well recognised and do not require discussion.

10. SUMMARY.

The insect exhibits partial adaptation to a temperate climate, the egg and adult being resistant to considerable cold.

The wide range of its food plants is indicated.

The adults are gregarious and show marked colour reactions. The life is long and the fecundity great. Parthenogenesis occurs and only male offspring result from this; mating is the rule and produces offspring of both sexes.

The incubation period of the egg varied from 8 to 117 days according to temperature, and the duration of the scale stage from 17 to 43 days.

The occurrence of *A. sonchi* Kotinsky in England was noted.

The attacks of the pest on tomatoes mainly make it of great economic importance.

Specialisation in tomato growing to the exclusion of other crops is a useful precaution and other precautionary measures are indicated.

Fumigation is the only effective method of treating infested plants. Naphthalene and tobacco preparations give little relief. Tetrachlorethane is a good fumigant, but is too costly for trade growers.

Cyaniding is the best method of treatment. The dose of sodium cyanide varies from one-quarter to one-tenth ounce per thousand cubic feet of greenhouse space, according to the type of greenhouse and the condition of the plants. Long fumigations with these small doses are more effective than short fumigations with larger quantities.

The precautions necessary to avoid damage to the plants are given, avoiding daylight during fumigation and having the roots of the plants dry being the most important.

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EXPLANATION OF PLATES I AND II.

PLATE I.

Fig. 1. Underside of leaf of zonal geranium showing circles of eggs of *A. vaporariorum.*
Fig. 2. *A. sonchi* Kotinsky, on *Sonchus oleraceus* in a Lea Valley tomato house.
Fig. 3. Three tomato plants cyanided side by side and photographed a fortnight after the fumigation. The hard plant was not damaged while the two soft plants show a severe scorch and crinkle of the foliage, the damaged leaves continuing to function partially. The plants are growing away from the damage.

PLATE II.

Two tomato plants photographed immediately before (Figs. 4 and 6) and a week after (Figs. 5 and 7) cyaniding together, $\frac{1}{4}$ oz. cyanide per 1000 c.ft., duration 9 hours, temperature 60–54° F., relative humidity 97 per cent. Fig. 4 represents a plant well watered and turgid, and the severe damage it received from the gas is shown in Fig. 5. Fig. 6 represents a plant which was not flaccid but required water, and Fig. 7 shows that it was undamaged.

(*Received July 27th*, 1921.)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 6.

Fig. 5.

Fig. 7.

OBSERVATIONS ON THE ENSHEATHED LARVAE OF SOME PARASITIC NEMATODES.

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(With 1 Text-figure.)

CONTENTS.

INTRODUCTION.

At the suggestion of Professor R. T. Leiper I took up the investigation of the larvae of certain parasitic nematodes. *Graphidium strigosum* and *Trichostrongylus retortaeformis*, occurring in the alimentary canal of the rabbit, were selected because the host is a convenient laboratory animal and because the parasites are of some economic importance in causing disease amongst wild rabbits. Moreover, both worms belong to the order of Trichostrongylidae of which many members are parasitic in the alimentary tract of cattle, sheep and other domesticated animals.

One of the chief objects of the research was to discover whether the ensheathed larvae of these two species can infect through the skin in a like manner to the ensheathed larvae of *Ancylostoma duodenale*, *Necator americanus*, *Strongyloides stercoralis* and some others. The work on *N. americanus* recorded in the following pages arose from the necessity of conducting experiments with larvae known to be skin penetrators. With regard to the location of the two rabbit parasites, *G. strigosum* occurs only in the stomach, whilst *T. retortaeformis* is found mostly in the small intestine but may occasionally occur in the stomach also. The former is reported only from rodents, but the latter has been found in sheep and goats in addition to rodents.

The adult females of all three species produce eggs which pass out in the faeces of the host.

MATERIAL.

Through the kindness of Dr C. F. Druitt of Alvaston, Derby, I was able to obtain a supply of material for this research. Dr Druitt has the shooting rights of some fields where the rabbits were noticed to be suffering from certain wasting conditions towards the end of 1920. One or two specimens of these diseased rabbits were sent to this department and were found to contain large numbers of the two parasites in question.

In the early part of this year, 1921, I got into touch with Dr Druitt and he very kindly supplied me with rabbit droppings from the same areas where the wasted rabbits had occurred. At another time he sent three live rabbits, two of which turned out to be heavily infected with *G. strigosum* and *T. retortaeformis*, and furnished a good supply of droppings containing eggs, until they died after being in captivity for a short time. On opening these rabbits large numbers of both parasites were found.

I should like to express my best thanks to Dr Druitt for the interest he has taken in the work and for the great assistance he has given in providing material.

The larvae of *N. americanus* were obtained in cultures from the faeces of one of the patients in the Hospital for Tropical Diseases.

CULTIVATION OF THE LARVAE.

By teasing out a few droppings from an infected rabbit in water it was easy to find the eggs of both *G. strigosum* and *T. retortaeformis*. In the case of mixed collections of droppings from the infected area, which have been used in this work, it was possible to recognise the eggs of both worms. Identification was an easy matter also, in that a good supply of adult worms was available, and these furnished the necessary eggs for comparison and measurement.

A number of rabbit droppings were broken down in distilled water until a fairly thin mixture was obtained. This was put into a Petri dish in a shallow layer. The lid of the dish was provided with a layer of clean blotting-paper which was kept moist, and afterwards the dish was put into the incubator at 22° C.

Numerous rhabditiform larvae developed in this culture, but became quiescent on the bottom of the dish after about two days and failed to develop further. This was probably due to the presence of toxic substances in the culture, so recourse was had to Looss's recommendation

of the use of animal charcoal. The next cultures were therefore put up with a liberal admixture of this substance with the teased-up droppings so that a moist, not sloppy, medium was finally obtained. This was put into Petri dishes, a shallow layer in each, and the lid of each dish was provided, as before, with clean, moist blotting-paper. The dishes were incubated at 22° C.

From these cultures large numbers of ensheathed larvae of both *G. strigosum* and *T. retortaeformis* were obtained in the course of six days.

The development of the larvae is in every way similar to that of *Ancylostoma duodenale* described by Looss(4) and of *Haemonchus contortus* described by Veglia(9).

Fig. 1. Tails of ensheathed larvae of A. *Graphidium strigosum*, B. *Trichostrongylus retortaeformis*. ×350.

On the hatching of the egg a typical rhabditiform larva is produced, having a buccal cavity, the walls of which appear under the microscope as two refractive rods followed by two dots. This is followed by the oesophagus which, for the greater part of its length, is somewhat cigar-shaped, and is then constricted into a much narrower portion, and finally swells out into a bulb within which can be seen the characteristic Y-shaped lining. The intestine succeeds the bulb and presents a wavy outline amidst the granular contents of the intestinal cells and terminates in the anus. After the first ecdysis the larva grows and the oesophagus loses its original appearance, becoming longer and without such pronounced demarcation into distinct regions. The posterior bulbous portion seems to become elongated and at the same time flattened. The cells

of the intestine become densely crowded with reserve food granules which sharply set off this region from the rest of the body.

Finally, at the end of this stage of growth, the larva becomes ensheathed by the replacement of its cuticle by a new one underneath. The mouth and anal apertures close up, and at the same time the enclosed larva shrinks a little in size so that it becomes separated from the old cuticle which encases it as a completely closed sheath. It then wanders upwards from its surrounding medium.

The ensheathed larvae of *G. strigosum* and *T. retortaeformis* are similar to each other in all essential structures. The former have much longer tails than the latter, and by this means can be easily recognised in a mixture of the two kinds (Fig. 1).

ATTEMPTS AT SKIN INFECTION.

(1) A large number of ensheathed larvae were collected from the culture dish lids containing moist blotting-paper by pouring distilled water on to the latter and allowing this to stand for a short time. These were concentrated in a small quantity of water by centrifugalising. A drop of this liquid was placed on the skin of a young rat (10 days old), in the inguinal region, and allowed to remain there for 20 minutes, the animal being held in position during this time. The water was not allowed to dry up, a drop being added to replace that lost by evaporation. It was then killed and the portion of skin was dissected out and fixed in 10 per cent. formalin. This was embedded in paraffin and sectionised, but the sections showed no sign of skin infection.

(2) The day following this experiment a large number of larvae in water were placed in the bottom of a glass tube, and into the liquid the hind-quarters of another young rat were immersed. The larvae could be seen moving actively in the liquid when examined through a hand lens. The animal was kept in the tube for one hour, during 20 minutes of which the tube was in the 37° C. incubator. After this the rat was chloroformed and the skin from the tail and both hind feet was fixed in 10 per cent. formalin. It was noticed that the larvae left in the tube after taking out the rat were very sluggish in movement and many were practically quiescent. I associated this with the fact that they had been put into the 37° C. incubator and inferred that the increase in temperature had checked their motility. Some of the larvae were placed on a slide in a drop of water and covered with a coverslip; they then became quite active again. This was no doubt due to the return to normal room-temperature as many subsequent observations proved.

Examination of the sections from the tail and one of the feet failed to reveal any sign of skin infection.

(3) A young mouse (body about 1 inch long) was chloroformed and the skin from the abdomen and flanks removed. This skin was stretched and pinned over a hole about $\frac{1}{2}$ inch in diameter in the centre of a piece of sheet cork $\frac{3}{16}$ inch in thickness. The cork was then floated on the surface of some *N* saline which had previously been warmed up to 37° C. and placed in a glass jar 3 inches high by $2\frac{1}{4}$ inches in diameter, having a well-fitting stopper. The cork floated near the top of the jar and care was taken that the warm saline came into contact with the underside of the skin by first allowing the bubble of air to escape from between the skin and the cork. The jar could be placed on the stage of the binocular dissecting microscope and the surface of the skin easily examined.

A drop of water containing a large number of active larvae was placed on the skin. Immediate examination showed them to be actively moving in the drop. The jar was placed in the incubator at 37° C. and examined every quarter of an hour during a period of one and a half hours. During this time some of the larvae could be seen moving very sluggishly amongst some threads of blotting-paper in the drop, whilst others moved more actively. As far as could be seen there was no down-boring motion exhibited by the larvae and no sign of skin penetration. At the end of an hour and a half the bulk of the supernatant water of the drop was drawn off by means of a capillary pipette, and a drop of fresh white of egg was placed on the skin. Examination under the microscope showed the larvae moving in this. The albumen was then coagulated by dropping hot 90 per cent. alcohol on to it from a pipette. This was done so as to fix in position the larvae which had been added with the original drop of water. The skin was then immersed in 10 per cent. formalin for fixation. It was later on treated in the usual way and embedded in paraffin for sectionising. Examination of the stained sections failed to reveal any sign of skin infection, though many slides showed sections of larvae between the coagulated albumen and the epidermis.

I realised that in the above described experiments I had, quite possibly, not brought about the conditions requisite for the larvae to penetrate the skin, *i.e.* conditions under which known skin penetrators such as the larvae of *Ancylostoma duodenale* or *Necator americanus* would act. I therefore determined to obtain a culture of the eggs of one of these forms and rear a number of the ensheathed larvae with a view to finding out the exact experimental conditions required by these for skin penetration.

In due course a stool was obtained containing a large number of adult *N. americanus* together with their eggs. Cultures of the faecal matter were put up with animal charcoal in large Petri dishes the lids of which were provided with a layer of blotting-paper, which was always kept moist. After eight days a good supply of ensheathed larvae was collected from the lids by washing the blotting-paper with water. The washings were centrifugalised and the larvae concentrated into a small bulk of water.

A young rat, three days old, was chloroformed and the skin from the abdomen and flanks was removed. This was stretched over a hole in a piece of sheet cork and pinned in position. The cork was then floated on the surface of *N* saline warmed to 37° C. contained in a glass jar as already described.

Examination of the drop under the microscope showed the larvae in very active movement with their anterior ends pressing downwards on to the skin as though trying to get into it. The jar was closed by its stopper and placed in the incubator at 37° C. At frequent intervals it was taken out and after removal of the stopper the drop was examined under the microscope. Always the larvae were seen to be very actively wriggling in a downward direction, but at the end of two hours there was no sign of any of them having escaped from their sheaths and penetrated the epidermis. I did not understand the reason for this as I was under the impression that I had brought about the requisite conditions for skin penetration. However, I put the jar with the cork and its attached skin back into the incubator, but with the stopper left out. On examining the preparation under the microscope the following morning I found that the drop of water had evaporated and that on the surface of the skin, within which many larvae could be seen, there were several empty sheaths. From this I inferred that evaporation of the water containing the larvae was necessary before they could leave their sheaths and penetrate the skin. At all events it seemed probable that there was some mechanical necessity for a shallow drop or even a film of water rather than a globule for the larvae to act in. In a deep globular drop, although they could be seen actively wriggling downwards on to the skin, they seemed to lack the necessary purchase of pressure upwards against a resistant surface to enable them to leave their sheaths and penetrate the skin[1].

[1] Looss's description ((4), p. 431) of his repetition of Herman's experiment on the effect of methyl-green stain on ensheathed *Ancylostoma* larvae bears on this point. He found that if the drop containing the larvae and the stain was not covered with a coverslip

The day following this experiment two more were set up. In one I placed a drop containing many active larvae on the surface of a piece of skin taken from a young rat's abdomen as on the previous day and under the same experimental conditions. The jar with its floating raft of cork carrying the skin stretched over the hole with the saline in contact with the lower surface of the skin was placed in the incubator at 37° C. but the stopper was out of the jar. Examination showed the larvae in active movement, wriggling downward, their anterior ends pressing against the surface of the skin. Several examinations were made during the course of practically two hours and each time the larvae could be seen actively in motion. The last time the preparation was examined, *i.e.* after 1 hour and 55 minutes, it was found that the drop had completely disappeared and, owing to the conditions of illumination, it was difficult to make out the details of the surface of the skin. I therefore placed a drop of clean distilled water on the surface of the skin. There could now be seen a large number of empty sheaths and one or two freely moving larvae. Practically all the larvae, however, had left their sheaths and penetrated the skin, where they could be seen moving slightly when the preparation was suitably illuminated. The drop of distilled water was removed by means of a pipette, and when examined later on was found to be rich in empty sheaths. The skin containing the larvae within it was fixed in hot 70 per cent. alcohol and the next day a portion of it was divided into two layers, for the epidermis and the immediately subjacent layers separated very easily from the dermis, and the upper epidermal layer was cleared in lactophenol and mounted whole. In this way a most interesting slide was obtained showing empty sheaths on the actual surface of the skin whilst just below, embedded in the epidermis, could be seen numerous larvae which had penetrated the skin.

In the other experiment, which ran concurrently with that just described, I used a piece of skin from the back of a young rat. The skin was pinned on to a sheet of cork as before, and instead of putting the drop of water containing the larvae directly on the skin, I placed on the latter a small piece of clean blotting-paper. This was done to imitate in a miniature way Looss's ((4), p. 519) experiments, in which he applied a pad of sacking or gauze to a dog's skin and then put the active

the larvae could not get out of their sheaths. They required the presence of a downward pressure of the coverslip before they could obtain the necessary purchase to break the anterior end of the sheath open and then creep out. I repeated this experiment with *Necator* larvae and obtained the same result.

Ancylostoma larvae in suspension on the surface of the pad, giving them two hours in which to bore through the material and get into the skin underneath. I ensured that the blotting paper had good contact with the skin below, and then applied a suspension of active larvae to its upper surface. Immediate examination under the microscope showed the larvae actively wriggling on the blotting-paper. At the end of two hours no larvae were to be seen, and the blotting-paper was removed and put into a drop of distilled water for subsequent examination. The skin just beneath the blotting-paper showed two or three larvae moving on it, but it was impossible to see into the skin because it was too dense and rather pigmented. The preparation was placed entire into hot 70 per cent. alcohol, and on the following day the epidermis was split from the dermis and cleared in lactophenol. When mounted on a slide it was found that numerous larvae were embedded in it.

The water in which the pad of blotting-paper was placed was found to contain numerous empty sheaths. No active larvae emerged from it, thus showing that all had passed through it to the skin.

I have described these experiments in some detail because they reveal a convenient and easily manageable method of experimentation for skin infection work.

I next proceeded to use this method with the ensheathed larvae of *G. strigosum* and *T. retortaeformis*. A young rat, seven days old, was secured and chloroformed. The skin was removed from the abdomen and flanks and was found to be very soft and tender. It was stretched on a sheet of cork and pinned over the hole in the manner already described, and then the cork was floated on normal saline at 37° C. A drop containing numerous active larvae was placed on the surface of the skin, and it was at once evident, on examining the drop under the microscope, that the reaction of these larvae to the temperature of the saline, 37° C., was quite different from that of the *Necator* larvae. The latter executed lively downwardly directed movements as though trying to get into the skin, and were decidedly more motile at 37° C. than at laboratory temperature. The *G. strigosum* and *T. retortaeformis* larvae, on the other hand, very quickly became sluggish in their movements at the higher temperature. In fact they seemed to be upset and incommoded by the new conditions and made no downwardly directed movements. The stopper was left out of the jar and the latter was put into the incubator at 37° C. It was taken out at various intervals and the drop, which gradually evaporated, was examined under the microscope. It could then be seen that most of the larvae aggregated to

one side of the drop and coiled up, watchspring-wise, and remained quiescent. At the end of two hours only one or two could be seen moving on the surface of the skin, and these rather slowly and aimlessly.

The jar was left in the incubator overnight, and in the morning was put out on the laboratory table and left until it reached room temperature. It was then examined and the larvae were seen to be slowly moving about. Replaced in the incubator and left overnight again, it was examined on the following morning, when it was seen that most of the larvae were still crawling about and had evidently become accustomed to the higher temperature. The skin was split into two layers, as had been done in the *N. americanus* preparation, and examined under the microscope. It was then easy to see that the larvae were still ensheathed and had made no attempt to penetrate the epidermis after contact with it for 48 hours.

The foregoing experiments give no evidence that the ensheathed larvae of *G. strigosum* and *T. retortaeformis* are capable of penetrating through the skin.

Veglia ((9) p. 424) in his paper on *Haemonchus contortus* gives an account of his attempts to get the ensheathed larvae of this worm to infect through the skin. His final test of whether infection had taken place was to search for the adult worms in the intestine or the presence of eggs in the faeces. In one case larvae were injected under the skin, but though these remained alive for a short time they did not set up an infection. In all cases he failed to secure infection through the skin, so that my experiments fully support his negative results.

Theiler and Robertson ((8) p. 321) placed large numbers of ensheathed larvae of *Trichostrongylus douglassi* on the skin of an ostrich to test the possibility of skin infection. The bird used was kept under close observation for eight months but at no time were the faeces found to contain eggs.

Brumpt(2), making use of human umbilical cord, though the method of experimentation is not given, includes *Trichostrongylus* (*retortaeformis*?) (his query, not mine) from the rabbit in his list of larval forms which he says penetrate the cord tissue. The paragraph is as follows: "En nous servant du cordon ombilical humain, nous avons pu constater qu'il attire et se laisse pénétrer par les espèces suivantes: *Necator americanus*, *Strongyloides papillosus* du Mouton et du Lapin, *S. stercoralis*, *S. suis*, *S. vituli*, *S. sp.* d'un Macaque, *S. sp.* d'un Cercopithèque, *S. equinus* et *S. vulgaris* du Cheval, *Characostomum longemucronatum* du Porc, *Trichostrongylus* (*retortaeformis*?) du Lapin et par une larve d'un parasite du Mouton (*Chabertia*?)."

He meets the obvious criticism that these are not cases of skin infection by the following: "On pourra nous objecter que la faculté présentée par les larves infectieuses de certains Nématodes de pénétrer dans le cordon ombilical ne prouve pas qu'elles soient susceptibles de traverser la peau."

In view of my own results detailed above I have no hesitation in claiming that the ensheathed larvae of *T. retortaeformis* from the rabbit can be ruled out of the list of skin penetrators.

EFFECTS OF TEMPERATURE.

The behaviour of ensheathed larvae of *G. strigosum* and *T. retortaeformis* was so markedly different from that of *N. americanus* when applied to the skin at 37° C. that I determined to test the matter further.

For this purpose I used an electric warm stage in which a slide carrying a drop containing larvae can be placed and the temperature gradually raised from room temperature to 37° C. In this way I was able to watch the reaction of the larvae to the rise in temperature and to determine fairly well the optimum temperature for greatest activity. The result is shown in the accompanying table.

Graphidium strigosum and Trichostrongylus retortaeformis.

Temperature	Remarks
22–23° C.	Larvae showing good motility
23–24	Very active
24–25	Very active at edge of drop
25–26	1 or 2 showing coiling movement, rest actively motile.
26–27	A few showing sharp spasmodic backward and forward bending, rest wriggling actively
27–28	A few coiling and uncoiling, rest actively motile
28–30	Those coiled remained coiled longer, about half coiled, rest active
30–31	More than half coiled
31–32	A few moving actively, rest coiled and remaining so, 1 or 2 straight and motionless except for an occasional movement of one end
34	1 or 2 active, the rest coiled or straight and motionless
35·5	Only one moving, rest as at 34° C.
36·6	No movement

From the above it can be seen that the optimum temperature for greatest activity is between 22° and 25° C. At 25° C. coiling begins to take place and by the time normal body temperature is reached practically all motility has ceased.

This is a curious fact and not easy to understand when one remembers that the larvae require for their further development to get into the alimentary tract of the host where, of course, they will be permanently at 37° C.

They must, within quite a short period after ingestion, become accustomed to their new temperature and surroundings and resume fairly active motility in order to escape from their sheaths. It is possible that they require the specific stimulus of contact with their final environment, the stomach and intestinal walls respectively for *G. strigosum* and *T. retortaeformis*, for their emergence and further growth, though Veglia ((9) p. 426) has shown that the larvae of *Haemonchus contortus*, when taken by lambs with grass, can escape whilst in the mouth.

As bearing on this point I would refer to the observation recorded above, p. 40, in which I mention having found the larvae of *G. strigosum* and *T. retortaeformis* active after being in contact with the skin for nearly 48 hours at 37° C.

RESISTANCE TO DESICCATION AND EFFECT OF PLASMOLYSING SOLUTIONS.

Intimately associated with the power possessed by the larvae of *Necator* and *Ancylostoma* to penetrate skin is, I think, their lack of power to withstand desiccation. Looss ((4) p. 398) deals with the latter at length and says that "*Ancylostoma* larvae can remain alive on a surface which is becoming dry (and to which they adhere) so long as the envelopes surrounding them (the 'cysts' or 'sheaths') retain moisture within them. As soon, however, as this moisture begins to evaporate the bodies contract (generally in a longitudinal direction) and shrivel."

It is a very simple matter to allow a drop containing ensheathed larvae of *N. americanus* to evaporate gradually from a slide and in the course of my work I have performed the experiment a few times. It is difficult after the bulk of the water has dried up to make out the structure of the larvae, but it is very evident that they quickly feel the effects of desiccation even at room temperature, for if a drop of water is added to the slide after a few minutes, they fail to revive and resume active motion. None revive if the slide is allowed to remain dry for 15 minutes. It seems a natural inference to draw therefore that *Necator* and *Ancylostoma* larvae seek the protection afforded by penetration into skin because if they remained outside and became dry they would perish.

The ensheathed larvae of *G. strigosum* and *T. retortaeformis* on the other hand can withstand desiccation in the air for a time and when remoistened can revive and resume normal activity. Prolonged desiccation at high temperature and the action of direct sunlight are inimical to them as Veglia ((9) pp. 390 *et seq.*) has shown in the case of *Haemonchus*

contortus, but at ordinary room temperatures they can withstand air desiccation for a few days and revive on the addition of water.

Doubtless also the habit of coiling up watchspring-wise on the advent of desiccation and further of the tendency to congregate together as drying-up proceeds constitute additional safeguards to enable the larvae to withstand the adverse effects of desiccation. It is noticeable that when *N. americanus* larvae are dried on a slide they do not coil up after the manner of *G. strigosum*, *T. retortaeformis* and *H. contortus*.

The comparative rapidity with which *N. americanus* larvae can be killed, *i.e.* by a few minutes' exposure to dry conditions, points to the sheath being very permeable to water vapour and to the contained larvae being very easily injured by withdrawal of water from its tissues. I therefore carried out a series of experiments to test this, comparing it with *G. strigosum* and *T. retortaeformis* larvae at the same time. For this purpose I made use of solutions of common salt of different strengths brought into contact with the larvae, from 15–20 in number in each case, in shallow glass capsules, and noted the effect on the organisms through the microscope. The salt solutions—5 per cent., 10 per cent., 15 per cent., and concentrated—acted by withdrawing water through the sheaths from within outwards, and had the effect of bringing about a gradual cessation of movement and finally plasmolysed the contained larvae, causing vacuolations within them.

Records of the action were taken every five minutes and at the end of each test the power of revival was tested by transferring the larvae, after first washing them in a fair bulk of distilled water, to a capsule containing more distilled water.

Five per cent. saline causes *N. americanus* larvae to slow down their movements in 20 minutes, and at 35 minutes all are quiescent or quiet. *G. strigosum* and *T. retortaeformis*, on the other hand, remain normal in movement for 20 minutes, one or two began to coil at 25 minutes, and a few remained active even for 90 minutes. On transferring to distilled water after 2 hours in the saline, only five *N. americanus* larvae showed signs of revival and this not very complete, whilst the *G. strigosum* and *T. retortaeformis* larvae all revived and swam about well.

Ten per cent. saline. *G. strigosum* and *T. retortaeformis* larvae withstand the plasmolysing action of this strength longer than the *N. americanus* larvae. They also revived in distilled water to a much greater extent than *N. americanus* larvae.

Fifteen per cent. saline. As before in the lower percentages the *G. strigosum* and *T. retortaeformis* withstood the action and remained

capable of motility longer than the *N. americanus* larvae, and none of them revived in water. I performed two tests for power of revival for *G. strigosum* and *T. retortaeformis* larvae. In the first case I transferred them to water after $2\frac{1}{4}$ hours, *i.e.* after all motility had ceased, and then two or three revived after 2 hours. In the second test I transferred them to distilled water after 20 minutes in saline, *i.e.* after the period required to bring all movement to an end in *N. americanus* larvae. In this case all the larvae revived.

Saturated solution of saline. This causes cessation of movement very rapidly in all cases, and after transference to distilled water no *N. americanus* revived, but two of the others showed signs of movement.

It is clear from the above that *G. strigosum* and *T. retortaeformis* larvae are more resistant to the action of plasmolysing agents, and after the action of such are more capable of revival than the larvae of *N. americanus.*

I conclude from these results that the sheath in the case of *N. americanus* larva is more permeable than that of *G. strigosum* and *T. retortaeformis* and that the larva within is more easily injured by the withdrawal of water from its tissues than in the case of the other two organisms.

NATURE OF THE SHEATH.

It has already been pointed out that the sheath of all ensheathed larvae is produced by the replacement of the cuticle by the development of a new one underneath. It is well known too that the cuticle of all nematodes is composed of a very resistant substance capable of holding up most fixing agents and rendering the staining of these organisms a very difficult business. I decided therefore to carry out a few tests to obtain if possible a little more information as to the nature and properties of the sheath both in *N. americanus*, *G. strigosum* and *T. retortaeformis* larvae.

Martin (5) p. 101) quotes the results obtained by Lambinet and others with the ensheathed larvae of *Ancylostoma.* Lambinet found that corrosive sublimate 0·2 per cent. does not kill them, that 3 per cent. phenosalyl arrests their movements after $1\frac{1}{2}$–2 hours; Fernbach's liquor diluted 1 in 10 does not immobilise them after an hour; the pure liquor acts in $\frac{1}{4}$ hour. Five per cent. sulphuric acid kills after $\frac{3}{4}$ hour; a saturated solution of sodium bicarbonate does not kill after 2 hours, neither does Eau de Javelle after 1 hour. Camphorated petrol seems to stimulate their vitality; 3 per cent. lysol kills in 1 hour. Chloroform, ammonia and carbon disulphide all kill within 24 hours; formol vapour does not kill

after this length of time. Thirty per cent. saline and pure glycerine produce strong plasmolysis and kill the larvae.

Looss ((4) p. 439) quotes the results of Leichtenstern, Lambinet, Breton and Boycott, who all tested the resistance of ensheathed *Ancylostoma* larvae to the action of gastric juice, and found that the sheaths were not affected in any way by the peptic ferment.

These results show that the sheath is composed of a very resistant substance and Martin speaks of it as *chitinous* in character.

In my experiments I found that the sheaths of *N. americanus*, *G. strigosum* and *T. retortaeformis* are insoluble in the following reagents: water, alcohol, xylol, chloroform, phenol, lactophenol, formol, glycerine.

The sheaths remain unaffected even after several days' immersion in solutions of pepsin and trypsin, though the larvae within are ultimately killed and show signs of disintegration.

The sheaths are soluble in concentrated hydrochloric acid. Those of *N. americanus* become dissolved in the course of $1\frac{1}{2}$ hours at room temperature, whilst the sheaths of *G. strigosum* and *T. retortaeformis* resist the action for about $2\frac{1}{2}$ hours. It is of interest to note that chitin is soluble in concentrated hydrochloric acid yielding glucosamine.

Caustic soda, 5 per cent. solution, dissolves the sheaths and the enclosed larvae at 37° C. when left in the incubator overnight, whilst a 15 per cent. solution dissolves the sheaths of all three kinds within $1\frac{1}{4}$ hours.

The action of this alkali shows that the sheath substance is not real chitin since the latter is prepared from insects, etc., by the prolonged action of alkalis and repeated washings in water.

The sheaths stain easily and uniformly with 1 per cent. solution of methyl-green and fuchsin. I found that *N. americanus* larvae came out of their sheaths, as found by Herman and confirmed by Looss, when the drop containing the larvae and the stain is covered with a coverslip. *G. strigosum* and *T. retortaeformis* larvae did not exsheath. The sheaths also stain a little with picric acid solutions.

These results show that the sheaths are composed of some substance of a resistant nature, but not so resistant as true chitin obtained from various Arthopoda, Arachnida, Mollusca and Polyzoa, since they dissolve quite readily in 5 per cent. caustic soda.

SUMMARY.

1. The eggs of *Graphidium strigosum* and *Trichostrongylus retortaeformis* give rise, under suitable cultural conditions, to larvae which finally become ensheathed and wander from the culture medium.

2. A new and easily manipulated method of experimentation for skin infection work is described, in which skin from a young freshly killed animal, rat or mouse, is stretched over a hole in a piece of sheet-cork and pinned in position. The cork is floated on *N* saline at 37° C. and care is taken to ensure that the saline comes into contact with the underside of the skin. A drop containing the larvae to be tested is placed on the upper surface of the skin. The saline is contained within a suitable glass jar and the whole can be placed on the stage of a binocular dissecting microscope and the surface of the skin examined at any moment.

3. By this method it was found that ensheathed larvae of *Necator americanus* leave their sheaths and penetrate the skin when the drop of water containing them is allowed to evaporate and become sufficiently shallow to enable them to obtain a purchase against the surface of the drop. These larvae are very actively motile at 37° C.

4. Ensheathed larvae of *G. strigosum* and *T. retortaeformis* do not penetrate the skin under exactly the same experimental conditions. They are not actively motile at 37° C. but become coiled and quiescent at this temperature. Their temperature for optimum activity is shown to be between 22° and 25° C.

5. Ensheathed larvae of *N. americanus* cannot resist desiccation in air at room temperature and cannot revive on being moistened, whereas the larvae of *G. strigosum* and *T. retortaeformis* can withstand air desiccation and are easily revived on being remoistened. It is shown that the ensheathed larvae of these two species are more resistant to plasmolysing agents than the ensheathed larvae of *N. americanus*.

6. The sheath surrounding the larvae in all three species named in the foregoing paragraph is composed of a very resistant substance whose composition is not known. It is not true chitin since it is readily soluble in 5 per cent. caustic soda.

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LEAF CHARACTER IN REVERTED BLACK CURRANTS

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(With 46 Text-figures and 11 Graphs.)

It is well known that in marked cases of reversion in black currants the leaf undergoes considerable modification. It becomes relatively long in proportion to its breadth though generally of smaller surface than normal leaves. In general effect it bears a considerable resemblance to the leaf of the stinging nettle, and the disease for this reason is frequently known as "nettle leaf." Further, when beyond the youngest stages such leaves acquire a thicker texture and a darker colour and the serrations of the margin become fewer and coarser. There is indeed no great difficulty in identifying the disease in its advanced state. At the beginning of an attack, however, it is by no means so easy to identify. Growers usually put down such cases as "suspicious" or "going" and when pressed for their reasons say that they judge from the general appearance and not from any definite signs.

Such an absence of definite data is not only unsatisfactory in the field in view of the importance of propagation from absolutely sound stocks, but it limits the experimenter very much in attempting any experiments under controlled conditions. It is obviously important for him to be able to identify with certainty the initial stages and to have some means of marking the extent of the disease on a bush from year to year. Further, unless some such means be at hand he cannot follow the course of the disease during the season so as to trace any possible seasonal variations. The method to be described was arrived at by careful comparison of a normal with a reverted leaf. It has the advantage of being a numerical one and therefore independent of the general opinion of the observer as well as of the size and shape of the leaf.

If any leaf be looked at from the undersurface, it will be noticed that there are five main veins arising from one point at the extreme base of

the leaf (Fig. 23). These veins run to the five main points of the leaf, *A–E*. Now if the submain veins arising from the midrib on one side and running to points on the margin (neglecting, of course, to count the main veins to *B* and *D*) be counted, it will be found that they number at least five in a normal leaf. Sometimes there are six or seven, but never less than five. In a definitely reverted leaf, however, they are less than five, three being a common number in well-developed cases (Figs. 22*a*, 28, 29) and in extreme cases they may be reduced to zero (Fig. 46, right-hand side). The second character to observe is the margin. In normal leaves (Figs. 23, 40) there are numerous fine serrations, many of which do not receive any submain branches, but are innervated from branches of a lower order. In reverted leaves the margin has comparatively few and coarse serrations (Figs. 27–29) and only a few fine serrations exist which receive veins of a lower order than submain.

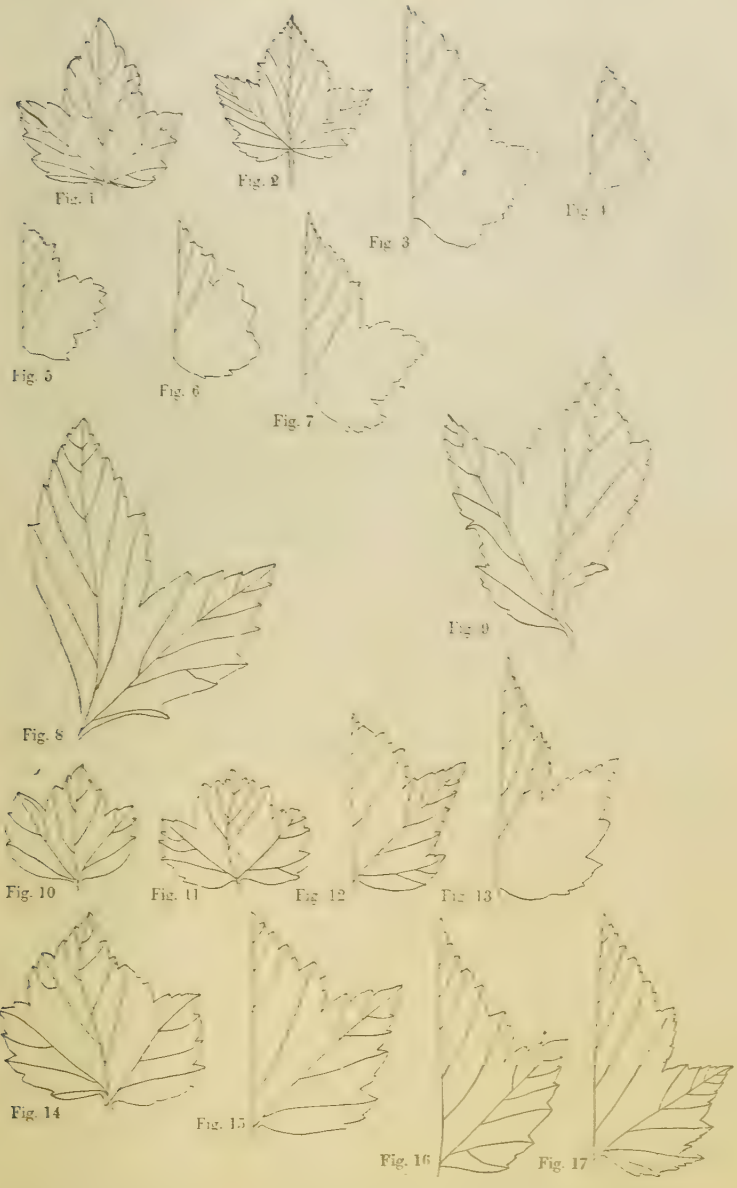
These two numerical indices have been found extremely useful both in the laboratory and in the field for exact work and immediately reveal the fact that reverted leaves are sometimes large and broad (Fig. 36), small and broad (Fig. 22*a*), or entirely irregular and deformed (Fig. 31). It also shows up the fact that small and comparatively pointed leaves need not necessarily be reverted (Figs. 2, 20).

TEMPORARY REVERSION.

Using these two methods it has been possible to study certain cases of what may be properly called "temporary reversion" which have come under the writer's experience.

Case 1. This case occurred in a pot plant kept in a greenhouse. The plant was an oldish one and in poor condition. It possessed two shoots *A* and *B*, both of which were made up of two or three years' growth. The two and three year old wood was bare of buds and of the usual blackish colour common to old black currants. Each also possessed about nine inches of one year old wood covered normally with buds.

In early spring the shoot *A* was ringed just below the one year wood. The operation was performed in the usual way, a ring of tissue about three-sixteenths of an inch wide as far as the cambium being removed. This operation was performed for an object quite apart from reversion, but it produced a very interesting effect. As usual the buds below the ring, in this case dormant ones, were forced into growth, but the leaves instead of being normal were reverted as judged by the venation and margin tests. They were not particularly narrow in shape but had a generally reverted appearance. The other shoot *B* did not break from



Figs. 1–17. Temporary Reversion. Fig. 1. Reverted leaf from "ringed" shoot. Fig. 2. Normal leaf from wood previously bearing reverted leaves. Figs. 3–7. Case 2 of temporary reversion. Figs. 8, 9. Abnormal, deformed leaves resulting from interference to terminal bud. Shoot A, case 3. Figs. 10–13. The four basal leaves of Shoot B, case 3. Figs. 14–16. The first three basal leaves of Shoot C, case 3. Fig. 17. 6th leaf of Shoot C, case 3.

the older wood but produced normal though rather small leaves from the one year old wood. A few weeks after, a similar ring was made just below the one year old wood of *B*, but at first it produced no result. The whole plant at this time was suffering rather severely from the combined effects of aphis and a too hot and dry atmosphere with the result that the foliage became brown and dropped off and the plant took on a resting condition as in winter. Being of no further use apparently, it was put outside. After a few weeks the heavy summer rains caused the plant again to put out leaves, this being the second time during the season. This time, however, shoot *A* which had previously produced reverted leaves from the old wood showed perfectly normal leaves (from the buds formed in the axils of the first crop of reverted leaves). The portion above the ring had died. Shoot *B* this time produced reverted leaves from the dormant buds on the old wood in the same way that shoot *A* had done earlier in the season. The top portion of shoot *B* above the ring had also died. Outline drawings from a leaf of the second crop of shoots *B* and *A* are shown in Figs. 1 and 2. These two shoots therefore had reversed their behaviour, *A* producing first reverted leaves and then normal and *B* producing first normal and then reverted. Since they both belonged to the same plant it is clear that it could not be reverted in the ordinary disease sense.

Case 2. In order to test the effect of cutting back during the summer season a bush growing in the open had about three inches of growth cut away from every growing tip. (The original idea was to test the character of the foliage issuing from the weak lateral buds immediately below the pruning cut.) Owing to the lateness of the season very little growth occurred. This was, however, sufficient to show that the leaves produced under these circumstances were of the reverted type. Luckily, a shoot in the middle of the bush had been overlooked. This shoot had almost, though not quite, ceased growth in length and the stimulus placed upon it by removal of the active growing points from all the other leaders caused it to react in a very interesting manner. Fig. 3 shows a half outline drawing of the leaf that had just been formed before the stimulus began. It has fine submain veins and is almost normal in margin. The next leaf (Fig. 4) has lost a vein, the margin has become irregular and the leaf undersized. The next leaf (Fig. 5) is similar though somewhat larger, while in the next (Fig. 6) the veins have been reduced to three. The last in the series (Fig. 7) has regained the five veins and has become normal again in margin and outline. This therefore is clearly a case of temporary reversion.

Case 3. This case occurred in a cutting bed. When found, the single shoot arising from the cutting had divided (during the summer) into two shoots, one longer and one shorter. The reason for the division could not then be ascertained, but the original terminal growing point had absolutely disappeared and growth had been taken up by lateral buds immediately below. In Table I are found details of the important points. These are the venation of the leaf, the mites found in the axillary buds and the leaf margin and shape. The whole plant was free from mite infection at the time of examination. Characteristic temporary reversion is shown by the basal leaves of both longer and shorter shoots (*B* and *C*). In shoot *B* the two basal leaves (Figs. 10 and 11) have only three submain veins but a recovery is quickly made to five in leaf 4 (Fig. 13) and maintained to the end. Recovery of the margin is slower. Shoot *C* shows much the same transition. The first two leaves (Figs. 14 and 15) though broad are markedly reverted. The leaf venation has recovered by leaf 3 (Fig. 16), but the margin not until leaf 6 (Fig. 17).

The effects of the check to terminal growth, however, are not confined to the two shoots *B* and *C*; it has also marked influence on the undivided base of the cutting, here called shoot *A*. Normality of veins and margin is maintained up to leaf 9, namely five leaves behind the critical point. Leaf 10 was very small, deformed and of a general reverted type. Leaf 11 was unusually large but quite normal as if extra food supplies had been diverted into it. Leaves 12 (Fig. 8) and 14 (Fig. 9) were both peculiar, first in having no bud in their axils and secondly in having lost one lobe of the leaf, in one case the left lobe, in the other the right. Both were rather reverted in margin and 14 (Fig. 9) was reverted in venation. Leaf 13 like leaf 11 was normal though of extra large size.

There is evidence here of considerable disturbance to normal growth. Leaf 10, which was the first leaf to feel the effect of the killing process going on in the then terminal, apparently had its food supplies cut off so that a very deformed and small leaf resulted. The food which should have been available for the developing terminal apparently went into leaves 11–14. Such a process may be often observed in brassica plants where the terminals have gone blind; the leaves immediately below, even if cotyledons, become large and dark green. No reason for the moment can be suggested why leaves 12 and 14 lacked one lobe nor why neither had an axiliary bud. Shoots *B* and *C* each began under the same stimulus that caused the extra large top leaves of shoot *A* and both showed temporary reversion of the leaves.

Table I. *Boskoop cutting showing disappearance of terminal buds during summer. The two laterals immediately below took up the growth. Examined August 27th,* 1920.

A.

	Leaves from base	Venation	Mites in axiliary buds	Margin of leaf
No.	1	Missing	0	
	2	,,	0	
	3	,,	0	
	4	,,	0	
	5	5 submain veins	0	Nearly normal
	6	5 ,,	0	,,
	7	5 ,,	0	,,
	8	5 ,,	0	,,
	9	6 ,,	0	Normal
	10	Deformed	0	Very reverted
	11	6 submain veins	0	Normal. Leaf very large
	12	5 ,,	No bud in axil	Reverted. Left lobe lost
	13	6 ,,	0	Normal. Leaf very large
	14	3+ ,,	No bud in axil	Rather reverted. Right lobe lost

At this point the shoot divided into two, no trace of the original terminal being left.

B. Longer shoot.

	Leaves from base	Venation	Mites in axiliary buds	Margin of leaf
No.	1	3 submain veins	0	Very reverted
	2	3 ,,	0	Very reverted. Larger
	3	5 ,,	0	Less reverted
	4	5 ,,	0	,,
	5	5 ,,	0	,,
	6	6 ,,	0	Normal shape and margin
	7	6 ,,	0	,, ,,
	8	6 ,,	0	,, ,,
	9	6 ,,	0	,, ,,
	10	5 ,,	0	,, ,,
	11	6 ,,	0	,, ,,
	12	5 ,,	0	,, ,,
			Terminal 0	

C. Shorter shoot.

	Leaves from base	Venation	Mites in axiliary buds	Margin of leaf
No.	1	2 submain veins	No bud in axil	Very reverted
	2	3 ,,	0	,,
	3	3+ ,,	0	Less reverted. Larger leaf
	4	5 ,,	0	Slightly reverted. Almost normal shape
	5	5+ ,,	0	Slightly reverted. Almost normal shape
	6	6 ,,	0	Normal end of season leaf
	7	6 ,,	0	,, ,,
	8	6 ,,	0	,, ,,
	9	6 ,,	0	,, ,,
			Terminal 0	

Case 4. This case did not come under the personal notice of the writer but was reported by a grower. The case consisted of a temporary reversion caused by the attack of Capsid bugs.

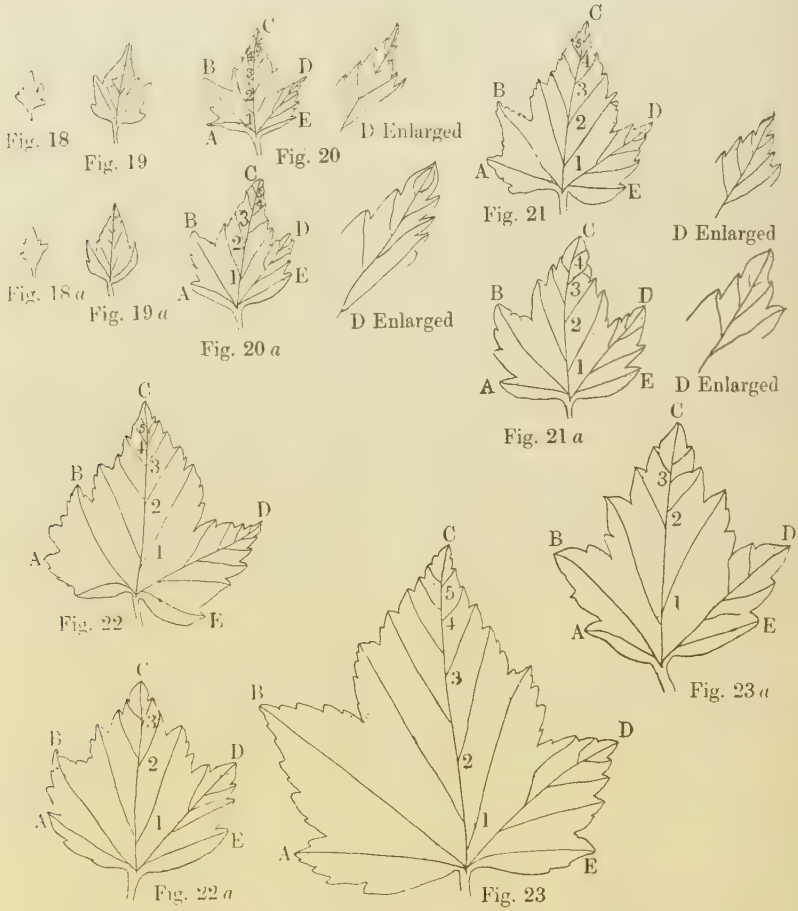
All these four cases seem to come under one general set of conditions. In the first three and probably in the fourth a considerable impetus to growth has been thrown on to weak buds. In case 1 dormant buds were stimulated by a ring above; in case 2 a bud that was just ceasing growth was suddenly urged into fresh growth by removal of all other active growing points and in case 3 weak laterals of the current season were acted on in the same manner. In the absence of exact physiological data it is impossible to make any sort of definite statement, but the conclusion that may warrantably be drawn from these cases is that the plant reacted under a special stimulus to growth.

DISEASE REVERSION.

Under this heading are grouped certain cases which have come under the writer's notice and which appear to be produced by causes other than those treated under the heading of "Temporary Reversion." They are of course similar in every way to those that appear in growers' plantations. The differences between normal and reverted leaves are perhaps most clearly brought out by a comparison of a normal and reverted shoot taking each, leaf by leaf, comparatively. Such a dual series is shown in Figs. 18 and 18*a* to 23 and 23*a*. In the two younger leaves (Figs. 18–19, 18*a*–19*a*) there are no marked differences but in the third leaves (20 and 20*a*) the blunter appearance of the lobes is already evident. In the enlargement of the lobe *D*, two points may be noticed. First the lobes are coarser, especially the apical one which is quite broad at the base in the reverted specimen and narrow in the normal one. Secondly the submain veins tend to become reduced in the reverted leaf, the top veins showing a characteristic bending round so that they nearly rejoin the submain vein instead of running to a point on the margin. These two characters are general throughout the leaf and constitute the best numerical means of ascertaining the extent of reversion. A still more advanced stage is shown in the enlargement of *D* of Figs. 21 and 21*a*.

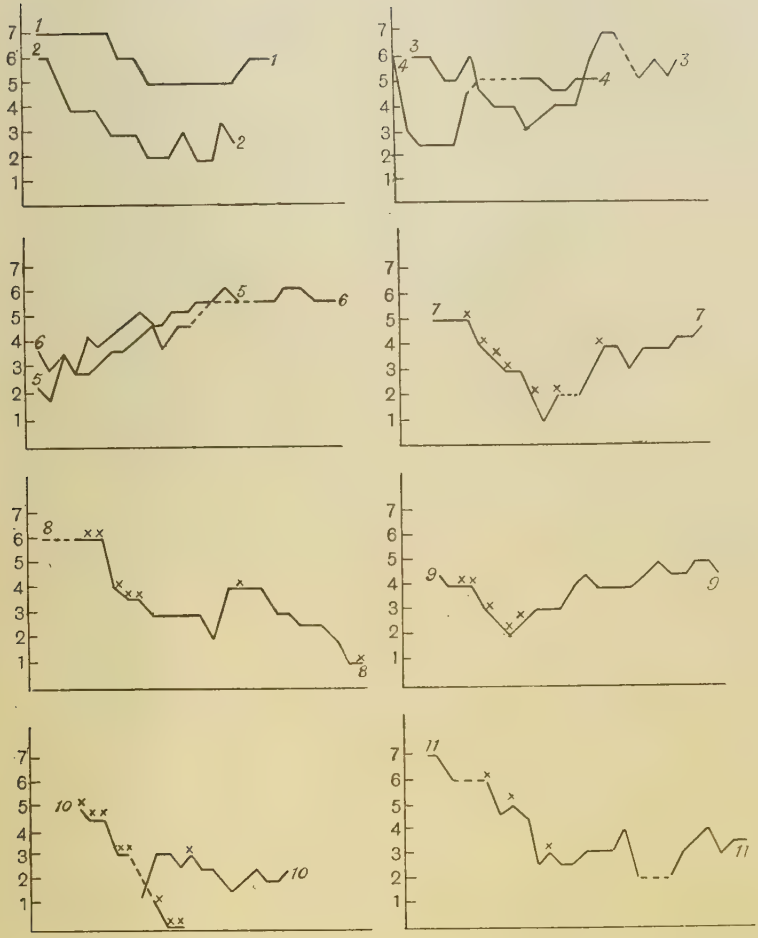
Figs. 21–23 and 21*a*–23*a* show successive stages in loss of submain veins from the midrib; 21*a* has four and 22*a* only three. These leaves were the fourth and fifth from the apex at the time of examination and are about the same size, but in the 10th leaves from the apex (Figs. 23 and 23*a*) a marked difference in size in favour of the normal has appeared. These may be taken as fair types of the two kinds. The changes that

have occurred may be summed up under three heads: (*a*) reduction of the venation system, (*b*) coarser and fewer lobes, and (*c*) reduction of leaf size in the older specimens.



Figs. 18–23*a*. Comparison of normal and reverted leaves. Figs. 18–22. First four leaves from apex of normal shoot. Figs. 18*a*–22*a*. First four leaves from apex of reverted shoot. Fig. 23. 10th leaf from apex of normal shoot. Fig. 23*a*. 10th leaf from apex of reverted shoot.

The following eleven cases have been selected from a number of similar ones from bushes growing at Long Ashton. The selection was made so as to reduce as far as possible the number of disturbing factors.



In each of the graphs 1–11, which correspond to the numbered cases considered in the text, the numbers in the ordinates indicate leaf vein numbers and the abscissae represent buds. Each graph starts from the basal leaf and proceeds upwards to the apex of the shoot. Where a leaf was missing the fact is indicated by a dotted line.

Table II.

1	2	3			4			5	6	
Veins	Veins	Veins	Mites	Leaf margin	Veins	Mites	Leaf margin	Veins	Veins	Mites
7	6	6	0	Normal	5	0	Normal	2+	4	0
7	6	6	0	„	3	3	Reverted	2	3	0
7	5	6	0	„	2+	0	„	4	4	0
7	4	5	0	„	2+	0	„	3	3	0
7	4	5	0	„	2+	0	„	4+	3	0
7	4	6	0	„	2+	0	„	4	3+	0
7	3	4+	0	Reverting	4+	0	„	4+	4	0
6	3	4	0	Reverted	Dam.	0	.	5	4	0
6	3	4	0	„	5	0	„	5+	4+	0
5	2	4	0	„	Dam.	0	.	5	5	0
5	2	3	0	„	5	0	Less rev.	5	4	0
5	2	3+	0	„	5	0	„	5+	5	0
5	3	4	0	„	5	0	„	5+	5	0
5	2	4	0	Rather less	4+	0	„	6	Dam.	0
5	2	4	0	Hardly rev.	4+	0	„	6	6	0
5	4	6	0	Normal	5	0	„	6+	6	0
5	3	7	0	„	5	0	„	6	Missing	0
6	.	7	0	„	5	0	„	.	6	0
6	.	Missing	0	„	.	0	.	.	6	0
6	.	5	0	„	.	.	.	.	6	0
.	.	6	0	„	.	.	.	.	6+	0
.	.	5	0	„	.	.	.	.	6+	0
.	.	6	0	„	.	.	.	.	6	.
.	.	.	0	.	.	.	.	.	6	.
.	.	.	.	.	.	.	.	.	6	.
.	.	.	.	.	.	.	.	.	.	0

7			8			9		
Veins	Mites	Leaf margin	Veins	Mites	Leaf margin	Veins	Mites	Leaf margin
Missing	0		6	10	Normal	Missing	Mod.	
„	BB		Missing	4	„	„	„	
5	BB	Normal	6	10	„	4+	„	Nearly normal
5	BB	„	6	6	„	4	0	„
5	BB	„	6	15	„	4	Mod.	Slightly rev.
5	0	Slightly rev.	6	0	„	4	BB	„
4	10	Reverted	4	0	Reverted	3	0	Very rev.
3+	0	„	3+	0	„	2+	0	„
3	BB	„	3+	0	„	2	0	„
3	0	„	3	0	„	2+	0	„
2	0	„	3	0	„	3	0	„
1	0	Deformed	3	0	„	3	0	„
2	BB	Reverted	3	1	„	3	0	„
Missing	0	.	3	0	„	4	0	Less rev.
2	0	„	2	0	„	4+	0	„
3	0	„	4	0	„	4	0	„
4	0	Less rev.	4	0	„	4	0	Normal in shape slightly reverted in margin
4	0	„	4	0	„	4	0	„
3	0	More rev.	4	0	„	4	0	„
4	0	Less rev.	3	0	„	4+	0	„
4	0	„	3	0	„	5	0	„
4	0	„	2+	0	Oak leaf	4+	0	„
4+	0	Semi-normal	2+	1	„	4+	0	„
4+	0	„	2+	0	„	5	0	„
5	0	„	2	0	„	5	.	„
.	0	.	1	0	„	4+	.	„
.	.	.	1	0	„	.	0	.
.	.	.	.	0	.	.	.	.

10			11		
Veins	Mites	Leaf margin	Veins	Mites	Leaf margin
Missing	Many		Missing	0	
„	Mod.		7	0	Normal
„	Many		7	BB	„
5	„	Slightly rev.	6	0	„
4+	„	„	Missing	Mod.	„
4+	„	„	„	0	„
3	„	„	6	0	„
3	„	Fully rev.	4+	BB	„
Missing		Second growth from here	5	0	Very rev.
1	„	Oak leaf	4	0	„
0	„	„	2+	0	„
0	Mod.	„	3	0	„
.	Many	.	2+	0	„
Second growth shoot			2+	.	„
Missing	0		3	.	„
1	4	Reverted	3	.	„
3	0	„	3	.	Not rev. Irregular
3	0	„	4	.	.
2+	0	„	2	.	Minute. First leaf of fresh growth
3	0	„	Missing	0	.
2+	0	„	„	0	.
2+	0	„	2	0	.
1+	0	„	3	0	Rev. margin but fairly broad
2	0	„	3+	0	„
2+	0	„	4	0	„
2	.	„	3	0	„
2	.	„	3+	.	„
2+	.	„	3+	.	.
.	0	.	.	.	.
.	.	.	.	0	.

They were all submitted to a more or less detailed analysis, notes being taken of the number of submain veins from the midrib running to points on the margin, of the mites present or absent in the corresponding axillary buds and of the character of the leaf margins (Table II).

Cases 1–6 were mite free, where examined for mite, with the exception of one bud in case 4, which for the moment may be regarded as negligible. Cases 7–11 were all affected with mite.

Case 1. This was a perfectly normal shoot. The seven first formed leaves, namely the basal ones, had seven submain veins, the next two six and then came a series of fives followed by three sixes. This shoot was examined on June 29th and had therefore by no means finished its growth. Graph 1 brings out the essential points more clearly. The drop from seven to five occurred largely during the month of June and possibly also during the end of May. This period, to judge by other growth graphs formed for apples and pears, constitutes the period of maximum growth. Now if, as previously indicated, a reduction of leaf veins occurs when the

plant is subjected to a special growth stimulus, then a similar reduction should be expected, though less in degree, when the plant is under the normal growth stimulus which occurs under optimum conditions of growth. This is apparently what happens. At the same time the veins were never reduced below five, which may be taken as the lowest figure for normality, nor was the margin in the least reverted.

Case 2. While case 1 illustrates a leaf series of a normal shoot, case 2 represents the same for a reverted one. The twig indeed came off the same bush but from the reverted half. In this case the basal leaves were normal with six veins, but the successive leaves showed greater and greater reduction of veins and increase of reversion until the figure two was obtained for the tenth leaf (Table II and Graph 2). At the end of the graph there is a slight tendency for the vein number to recover, but by June 29th the growth season had not nearly finished. The graph shows therefore a much more marked descent than in Graph 1, but of the same order. Here therefore there appear to be two factors working, reversion and the normal drop due to growth stimulus.

Case 3. This was a shoot from a cutting from a seedling. All the rest of the bush was normal. No mite could be found in any of the lateral buds or in the terminal. Reference to Table II shows that the reversion effect came on fairly suddenly at leaf 7 where there was a sudden drop to 4 – in veins and the margin first showed distinct signs of reverting. This continued till leaf 14 when the margin began to show signs of improvement and by 16 was practically normal again. The vein number follows in the same line. Graph 3 shows much the same condition as Graph 2 except that being examined later, on July 7th, more of the terminal rising portion of the curve was obtained. Here therefore is a shoot that started normally and finished normally but in between was strongly reverted.

Case 4. This was a shoot from a bush that had been cut back for grafting and the shoot came from the stock. Again here the first leaf was normal, but the reversion was very sudden. Bud No. 2 contained three mites, but except for this the shoot was completely free from mite infection. The recovery in leaf vein number was fairly quick, the normal figure of five being attained by the ninth leaf after which there were only very small variations. The margin recovery was neither so quick nor so complete, there being indication of reversion here even in the last leaf formed.

Cases 5 *and* 6. Both these were very strong shoots, case 5 being a shoot from the base of a bush cut back for grafting and case 6 a shoot

from a bush cut right down to the ground. Case 5 was not investigated for mite while none was found in case 6.

These two are considered together because their graphs (5 and 6) run practically together. Roughly speaking, only half the typical reversion curve, namely, the ascending half (of Graph 3), is represented. The reason that the first part of the curve is missed is probably this. Being hard cut back to weak buds growth starts relatively late in the spring and then is particularly vigorous owing to the upsetting of the balance between root and shoots. The late start also tends in the same direction, as general growth conditions are then fast approaching their maximum. However, the recovery of the venation is complete, in each case 6 or 6 + being attained. In one of these cases at least the issue was not directly affected by mite.

Case 7. In this and the following cases the position is complicated by the presence of mites in appreciable quantities.

In Table II the buds containing mites are indicated and an approximate figure given to indicate quantity.

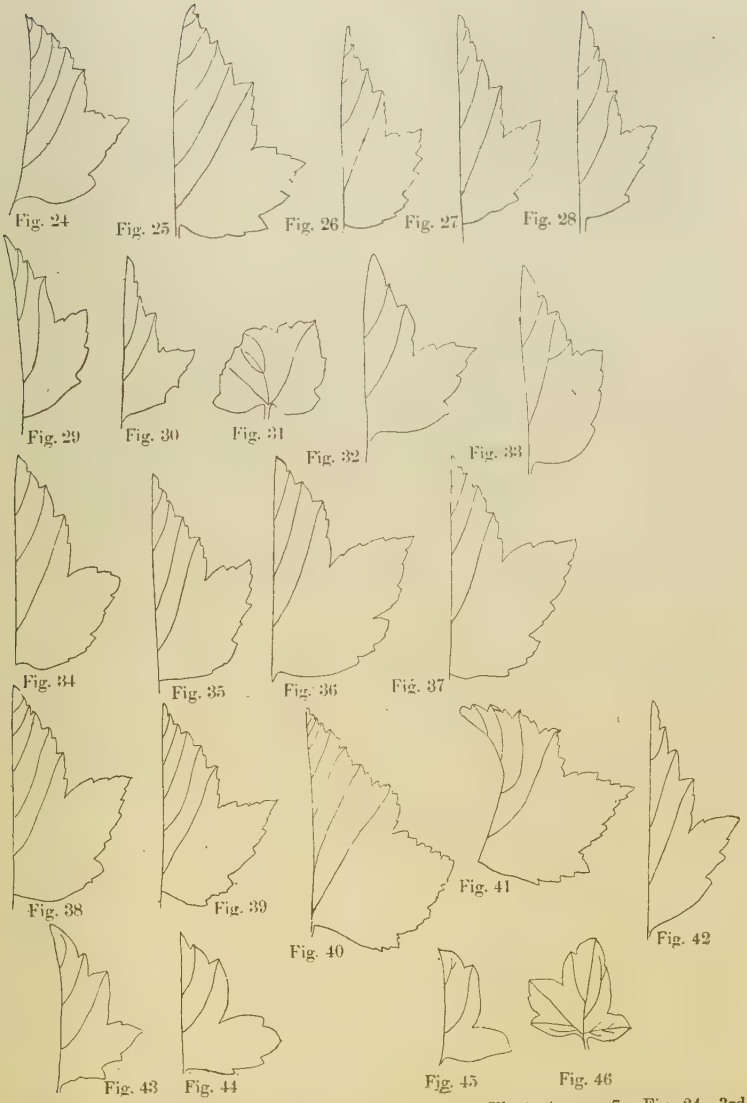
In the graphs mite attack is indicated by a ·, but the position has been shifted in each case to four buds in advance of the one actually affected.

In Table II, No. 7 it is seen that the most basal bud was mite free, and that the next four buds were fairly heavily infected. The veins remained five and the margin normal until the sixth which shows a slightly reverted margin and the seventh which shows a definite drop in vein number below normality, a drop which was subsequently continued. If the mite infection therefore has anything to do with the production of reverted foliage no effect was produced until the fourth leaf subsequently produced. For this reason in the graphs of 7–11 the position of the mite infected bud has been marked four leaves forward. After these four big buds only single mite free buds were found for a space of four more buds and reversion gets steadily more marked up to the twelfth leaf which has a venation number of one and a deformed appearance. The next leaf which is four leaves from the last big bud has begun to recover and subsequent leaves up to 18 continue this gradual recovery, the vein number by this time being four and the margin distinctly "less reverted." The leaf following shows a sudden drop of vein number to three and the margin becomes distinctly "more reverted." The following or 20th leaf has again suddenly made a recovery to four and "less reverted" and this recovery is continued until the end leaf which has a vein number of five and a practically normal margin.

The behaviour of leaf 19 calls for an explanation. It stands as an island of greater reversion in a sea of lesser reversion in the same way that bud 13 stands as an island of mite infection in a sea of mite freedom. The hypothesis immediately suggests itself that the two facts are connected and that the isolated mite infection has caused an isolated drop back into the more reverted state. In this case the two spots are six buds away, not four. It is of course impossible to lay down any fixed number of buds which would separate a mited bud from its possible effect on the growing point. In practice this has been found to vary between three and six, and four has been selected as an average to apply to the graphs. Referring to Graph 7 the crosses (which indicate mite infection) are generally followed by an increase of reversion. At first sight one might argue that as a drop is always experienced in mite free reverted twigs at this period (*cf.* Graph 3) so the drop in Graph 7 was due to this cause alone. On the other hand the drop is far more marked than in mite free cases, the vein figure reaching one, while three is usually the lowest reached in a mite free specimen.

However this may be, the isolated mite infection occurring in bud 13 stands in a portion of the curve that should be ascending and nevertheless an increase of reversion follows shortly afterwards. It distinctly suggests a close connection between mite infection and reversion.

These changes are illustrated in Figs. 24–39. Fig. 24 is the third leaf from the base. It is quite normal in every way. Fig. 25 is the sixth leaf where reversion is just appearing in the margin though the venation number is still five. Figs. 26–31 illustrate leaves 7–12 and show the gradual reduction of the veins to one and the gradual coarsening of the lobes of the margin up to Fig. 30; the nettle-leaf appearance of the whole leaf has also been gradually increasing. Fig. 31 shows the most reverted leaf of the whole series, and as has already been shown in certain cases of temporary reversion such leaves tend to be deformed. It suggests a very strong interference with the normal physiology of the plant. From this point to the 17th leaf (Fig. 35) reversion became less. Figs. 31–35 represent this initial improvement (leaf 14 missing). In Fig. 36, representing leaf 19, the leaf vein number has dropped to three again and the whole margin appeared more reverted. The differences in the margin may be more clearly shown by analysing the figures for the leaf on each side. Thus leaves 17 and 20 (18 was practically identical with 17) have each seven points between the apex and the sinus, four of which receive submain veins, while leaf 19 (Fig. 36) has only six, of which three receive submain veins. There is therefore a distinct numerical



Figs. 24–46. Mite infected Reversion. Figs. 24–39. Illustrate case 7. Fig. 24. 3rd leaf from base. Fig. 25. 6th leaf from base. Figs. 26–32. 7th–13th leaves from base. Figs. 33–35. 15th–17th leaves from base. Figs. 36–37. 19th–20th leaves from base. Fig. 38. 23rd leaf from base. Fig. 39. 25th leaf from base. Figs. 40–46. Series showing change from normal, through reverted, to oak leaf.

difference in addition to that readily perceived by the eye in the living specimen.

Unless the isolated mite infection in bud 13 be responsible for the sudden drop into the more reverted state there appears to be no reasonable explanation.

From this point onwards the recovery continues unchecked. The 20th, 23rd and 25th leaves are shown in Figs. 37–39, the latter being normal in vein number and nearly normal in margin.

Case 8. This was a reverted shoot from a half cut down bush examined on August 5th. The rest of the bush appeared normal. Here again, though the base of the shoot was mite infected, the first six leaves were in every way normal with a vein number of six. Reversion started quite suddenly at the 7th leaf on six buds in front of the first infected bud. The vein number suddenly dropped to four and the margin became reverted. Reversion gradually increased up to the 15th leaf with a vein number of two, after which a partial recovery to four set in for four buds, followed by further reversion to the end attended by the characteristic "oak leaf." As judged by the leaf vein number and margin, "oak leaves" appear to be merely an accentuated stage of reverted leaves. Such leaves (though not belonging to this series) are shown in Figs. 44–46.

The chief differences between case 8 and 7 are two in number. In Graph 7 the curve, with the exception of one bud, follows the general curve of reversion, the latter half having a general rising tendency. In Graph 8, though a short recovery begins at leaf 16, the general tendency of the latter part of the curve is downwards instead of upwards. The second point is that two isolated infected buds occurred after the basal infection. These were very slight as each only contained one mite.

Case 9. This was from an Ogden's Black bush with plenty of old big bud on the bush and was examined on August 6th.

This case shows in its graph the same sort of curve as does case 7, but there are no mite infected buds after the usual basal ones and the curve is of the same order as a reverted one unaffected by mites (of Graph 3). It is, however, different in that it drops lower, reaching a vein number of two, and also fails to reach so high a number at the end of the graph. Instead of attaining at this point a vein number of six or seven it scarcely reaches five and the leaf instead of being "normal" as in Graph 3 never gets beyond "normal in shape, but slightly reverted in margin." Though therefore of the same order as Graph 3 it is of different intensity and this difference may provisionally be referred to the mite infestation which is the only visible difference between the two shoots.

Case 10. This and the following one have been selected as special cases likely to throw light on the possible effect of mite infestation at the base. In both the shoot has ceased growth during the summer and a subsequent fresh growth has started from a bud later on. Case 10 was from a badly mite infested bush. It possessed five primary shoots of the current year and all contained mites in the terminal bud.

The particular shoot (10 in Table II) was examined on August 10th and bore twelve lateral buds, one of which, number nine, had subsequently grown out later in the season after the primary shoot had ceased growing. The graph for the primary shoot (Graph 10) showed a very quick descent to zero and reversion was so marked at the end that the leaves were strongly "oak leaf." If the hypothesis be accepted that mite infection produces reverted leaves, this was to be expected as the whole shoot was heavily infected. The secondary growth from bud 9 is shown in the lower half of number 10 in Table II. The first point to be noted is that bud 2 contained four mites. This was probably not a direct infection, but represented the residual mites in bud 9 of the primary shoot. It was probably due to the weakness of the infection that this bud grew out when growth conditions became urgent and not any other bud. The next point to be noted is that all the leaves are of the reverted type, though not so reverted as to become truly "oak leaf." The leaf vein number hovered about 2 + and showed no sign of a definite rise towards the end of the graph. The lower figure for bud 2 is probably due to temporary reversion due to growth conditions as indicated in the first part of this paper.

The conclusion that appears to be indicated is that this secondary shoot, though practically mite free and possessing none in the terminal bud, was under the influence of some special factor and that this factor was the high mite infestation of the primary shoot.

Case 11. This was from a young bush not heavily mite infected and examined August 31st. Its first seven leaves were normal with a high leaf vein number. Reversion began fairly suddenly at leaf 9 the margin showing up quite suddenly and the vein number quickly following in the next few leaves. Leaves 13 and 14 marked the bottom of the curve with a vein number of 2 −. From there, for four leaves the vein numbers and margin improved somewhat till at leaf 18 the shoot had ceased its first summer growth. As in the case of shoot 10, later on in the season a fresh growth began but this time it came from the terminal. This is of course the bud where one would expect a fresh growth, if anywhere, and no doubt it would have occurred from here in case 10

had it not been highly mite infected. In Table II, No. 11 a horizontal line is drawn at the spot where the first growth ceased.

Leaves 1 and 4 of the second growth shoot have the low vein number of two almost certainly owing to temporary reversion. Indeed, the whole shoot is not long enough to be certain that one has completely got rid of temporary reversion. On the whole the leaf vein numbers are on the low side though not so low as the secondary shoot of case 10. They indicate that they are still under the influence of the infected buds at the base of the primary shoot, but owing to the infestation being much less than in the case of the primary shoot of 10 the effect is also much less.

DISCUSSION.

These eleven cases may be divided into three classes, normal shoots as No. 1, reverted but mite free as Nos. 2–6, and reverted and mite infected as Nos. 7–11. With the exception of 5 and 6 all showed a vein number of about five (*i.e.* normality) at the beginning of growth. Both Nos. 5 and 6 were hard cut back shoots and these nearly always begin growth at a later period. Therefore it would appear that as a rule reverted bushes will commence the season with normal leaves.

Secondly all the shoots showed a drop in leaf vein number at the height of the growing season in May and June. The drop was slight in the normal shoots, well marked in non-infected reverts and still more marked in infected reverts. It would appear therefore that there are three factors at work, a seasonal one tending to reversion of a temporary kind, a reversion factor and a mite factor. One can of course never be sure unless one has carefully selected material that the fall in leaf vein number may not be due to the reversion factor only and not to the mite factor or that it may be due to both. In the mite infected cases above cited care was taken to select as far as possible only those shoots coming from bushes which otherwise appeared normal. Even supposing that all these cases would have proved reverted without mite infection, nevertheless the amount of fall is greater than in pure reversion cases and one may therefore presume that the mite infection has increased the effect. The same conclusion can be drawn from the behaviour of the ends of the graphs. Where the graph is long enough to show complete recovery the final leaf vein number is five or more for reverted cases (Nos. 3–6), but less where mite infection is not confined to the basal series (8). In 7 and 9 the recovery of the leaf vein number is practically complete, but the margin does not regain normality in either though it does in 3, the only reverted case with description of leaf margins completely free

from mite. In 4, provisionally included in the non-mite infected, the recovery of margin was not complete even though the mite infection was very small. Similarly in 10–11, also mite infected cases, the leaf margin never completely recovered. Evidence pointing in the same direction may be found on consideration of the relation of "oak leaf" to mite infection. In all such cases so far examined mite has always been found though not by any means always in the terminal bud. It seems quite clear that the effect of a massive infection of mite is conveyed by some means to the terminal growing point when this is itself quite free from direct infection. The oak leaf effect has never been observed by the writer so far in bushes where no mite has been found, though reverted leaves of the usual type are common on mite free shoots in certain cases. Now before oak leaves are produced the shoot always makes ordinary reverted leaves as in the series of figures in Figs. 40–46. One may therefore hazard the opinion that the same cause which produces oak leaf will, when acting in greater dilution, simply produce reverted leaves. So conversely, when one finds reverted foliage unassociated with mite (other than temporary reversion) there is at least an indication that either the same cause, namely mite, had been at work and the mite has for some reason disappeared, or the plant is still suffering from a residuum of previous infection. If these speculations have any basis in fact, and the writer does not put them forth except as speculations, then it would appear that reversion is largely quantitative in action. That is to say, the effect will depend more on the dose than anything else. This seems to be borne out in the cases examined. Where, as in 7 and 8, isolated mite infected buds occur a depression shortly follows in the graph, even when this should be normally ascending. The secondary shoot in case 10 appears to be suffering in the same way from the load of mite in all the buds of the primary shoot.

The conclusions above arrived at are emphatically only preliminary. The evidence supporting them is largely circumstantial and the reasoning is largely in the backward direction. This was for the moment impossible to avoid as no selected material was at hand, and no material could be safely selected until an effective method of diagnosing slight cases of the disease had been found. This, the writer maintains, is furnished by the leaf vein and margin method. It is now possible to select absolutely healthy material during summer for experimental purposes for the following season. It is hoped therefore that it will be possible to do direct experimental work under controlled conditions and so avoid the pitfalls hitherto unavoidable.

ABSTRACT.

1. A means is indicated whereby reverted leaves may be identified even in very slight cases or where the leaves have almost regained normality.

This method depends (*a*) on counting the number of submain veins running from the midrib to points in the margin, and (*b*) on observation of the margin points, which also may if necessary be reduced to a numerical basis.

2. That reverted leaves may be produced by artificial means, but that this reversion is of a temporary character. In each case examined the plant appeared to be under a special stimulus to growth.

3. Cases in the field were examined in detail in three respects, namely (*a*) leaf vein number, (*b*) mite infected buds, and (*c*) leaf margin. Three classes could be distinguished: (*a*) normal healthy, (*b*) simple reverted, and (*c*) mite infected (reverted). These corresponded with three factors which appeared to be acting: (*a*) seasonal factor, (*b*) reversion factor, and (*c*) mite factor. Furthermore, since "oak leaf" is an advanced stage of reverted leaf and is always associated with mite, the chances are that reverted leaves when found without "oak leaf" owe their existence in some way or other to the mite factor either patently or latently.

EXPLANATION OF TABLES I AND II

In the columns headed "Veins" the numbers represent the number of submain veins from the midrib running to a point in the margin. The veins on only one side are counted, but where an extra one appears on either side the sign + is used after the numeral. Similarly the sign + is used where the topmost vein is doubtful. Thus 3+ indicates either 3 on one side and 4 on the other or that on one side there were 3 clearly defined veins and one doubtful.

The words "Missing" and "Dam." indicate that the leaf was absent or damaged.

In the column headed "Mites" the approximate number of mites in the particular axiliary bud is given. "Mod." signifies a moderate amount and "BB" that a big bud was already forming, this being an indication of heavy infestation. The last number refers to the terminal bud. In the column headed "Leaf margin" the character of the leaf margin is shown in accordance with the description in the text.

(*Received August* 31*st*, 1921.)

FURTHER OBSERVATIONS ON *SITONES LINEATUS* L.

By DOROTHY J. JACKSON, F.E.S.[1]

(With 2 Text-figures.)

In a previous article(1) a full description was given of the attack of *Sitones lineatus* L. upon peas and beans, and it was shown that these plants, together with tares and lucerne, constituted the favourite food plants of this species; clover being little attacked when they were available. In the end of July, 1921, large numbers of adults of this species were found feeding upon clover and lucerne in Kent, and the following observations on the damage thus effected may be of interest to record.

Owing to the long drought during the summer of 1921, the second growth of clover in the hay fields had made little progress and the leaves were seriously attacked by adults of *S. lineatus* L. The leaves growing on many of the flowering shoots were eaten nearly to the midribs (Fig. 1, *B*), and the younger foliage at the base of the plants had also suffered severely (Fig. 1, *C*). Lucerne was similarly damaged. The attack was always most severe in those fields of clover and lucerne which adjoined fields of peas or beans. The latter were by this time cut and mostly harvested.

The young clover coming up amongst the corn was also much attacked (Fig. 2) many of the leaves being completely eaten off, especially along the edges of the field next to fields of peas and beans. Where the corn had already been cut the clover had made less growth and appeared to have suffered more from the attack of the weevils.

The adults of *S. lineatus* L. were abundant around the damaged plants. They were to be found during the day running about amongst the withered leaves and stalks that littered the ground at the base of the plants. Only a few of the beetles occurred upon the foliage in the daytime. As soon as darkness set in they crawled up the stems and commenced feeding upon the leaves, and numbers were captured in the sweep net at this time.

All were freshly emerged specimens, sexually immature. Not a single individual of the old generation was observed. Without doubt the vast

[1] A grant in aid of publication has been received for this communication.

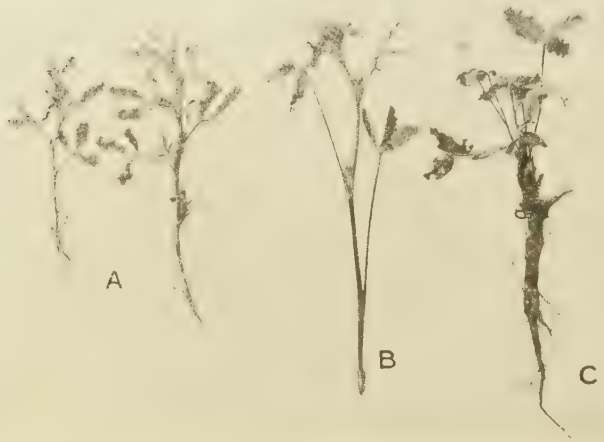


Fig. 1. Clover and lucerne with leaves partially destroyed by adults of *Sitones lineatus* L. *A* = lucerne (*Medicago sativa*), *B* = flowering shoot, and *C* = entire plant of red clover (*Trifolium pratense*) from aftermath of hayfield.

Fig. 2. Seedling plants of red clover with leaves destroyed by adults of *Sitones lineatus* L

majority of these beetles had been bred at the roots of peas, beans and tares and when these crops were cut had migrated to the clover and lucerne. It would appear unlikely that many of these beetles had been bred at the roots of the clover itself judging from the fact that the beetles are comparatively scarce upon clover in this district during the breeding season in spring, although they abound at this time upon peas, beans and tares, and also frequent lucerne. In the old pea and bean fields at the end of July many of the newly emerged beetles were still to be found amongst the stooks and when these were harvested a few remained under weeds upon the ground. They were also to be found amongst clover growing in permanent pastures and on waste ground. A certain number of other Sitones also occurred upon clover at this time. These included *S. puncticollis* Steph., *flavescens* Marsh, *sulcifrons* Thunb. and *hispidulus* F., species which (as will be shown in a subsequent article) live upon clover throughout the year and breed at its roots. Lucerne was also frequented by *S. hispidulus* and *S. crinitus* Herbst. These species contributed towards the general attack, but all were outnumbered by *S. lineatus* L.

REFERENCE.

(1) JACKSON, D. J. Bionomics of Weevils of the genus Sitones injurious to Leguminous Crops in Britain. *Ann. App. Biol.* VII, pp. 269–298.

(*Received September 18th*, 1921.)

CONTRIBUTIONS TO THE BIOLOGY OF FRESHWATER FISHES

By W. RUSHTON, A.R.C.S., D.I.C., F.L.S.

I. THE EFFECTS OF VARIOUS IMPURITIES IN A STREAM ON THE LIFE OF SPERMATOZOA OF TROUT AND YOUNG TROUT

At the suggestion of the owner of a salmon and trout stream in Banffshire, which of late years has become very polluted owing to various trade products, a series of experiments was undertaken to find out whether the various trade wastes have any effect on the fertilising power of the spermatozoa.

The method adopted was to have crude samples of every type of trade effluent collected when at its worst and delivered in London. On receipt of it the milt and ova were extracted from fully mature fish, and submitted to the various effluents in order to test the power of fertilisation in the presence of the impurity. When the milt and ova had been in contact a certain length of time the ova were placed in an artificial redd in running water and the number of eggs which hatched out and gave rise to normal embryos noted.

The whole of the work had to be done between December and March of the following year, as it is only in the late fall that fertile fish are obtainable. The trade effluents of the particular stream under consideration are waste products from six distilleries, consisting of spent malt products and yeast cells from the first distillations. Effluent from a tweed mill where wool-scouring, dyeing and weaving are carried on, and crude sewage from the town.

The effluent samples were collected as far as possible just as they were entering the stream except in the case of the cloth mills where this was impossible, owing to the mill standing over the stream, and only a diluted sample was obtainable.

The method of obtaining the ripe ova was to take mature brown trout and, after drying the fish as far as possible, by gentle pressure to extract the eggs into a clean dish: the same procedure was adopted in regard to the milt, great care being taken to prevent water mixing with

either milt or eggs till the two were brought in contact in the presence of the effluent under examination.

Ten eggs were taken up in each case and submitted to the various effluents with a measured quantity of milt and the two left in contact for 30 minutes to ensure uniformity throughout.

Some of the milt was examined microscopically at the same time and the length of time before activity ceased was noted.

It is known that the milt of trout will remain in good condition and fertilise ova 24 hours after extraction if kept free from moisture, *i.e.* if care is taken in extraction that no drainings or drippings from the male fish are allowed to come in contact with it; but if brought in contact with water they at once become very active and lose their fertilising power after three minutes' activity so that the period of activity is very short. This experiment can easily be done by taking a series of drops of milt on a slide side by side and adding the smallest drop of water to each and the time of activity taken.

It is further known that some spermatozoa of fish are more sensitive to poisons than young or mature fish and may be killed by such small amounts of poisons as can be detected only by very refined chemical methods.

Sample 1. Water from the upper reaches of the stream before any effluent had entered.

Colour	None.
Reaction	Neutral cold or hot using phenol-phthalein indicator.
Activity of sperms	Three minutes.

Eggs all fertilised and gave rise to normal embryos 90 per cent. of which hatched. One year and one two-year-old trout remained alive in this sample for over a week when they were removed to fresh water.

Sample 2. Containing effluent from one distillery.

Colour	None.
Reaction	Neutral hot or cold.
Sperms	Active for 2 to 3 minutes.

Eggs all fertilised and gave rise to normal embryos 90 per cent. of which hatched out.

Sample 3. Diluted sample from a woollen mill where wool-scouring takes place.

Colour	Slightly yellow.
Reaction	Neutral cold, alkaline hot.
Sperms	Active 2 minutes.

Ova fertilised and gave rise to normal embryos; 85 per cent. hatched out.

Sample 4. From same place as 3, but taken a few hours later; similar results to 3, but sample a little more alkaline. Two yearling trout introduced into these samples, although they showed uneasiness at first, soon settled down to the unusual conditions and were alive four weeks afterwards and during the period showed no unusual symptoms.

Sample 5. Taken from below the sewage outflow after crude sewage had entered the stream.

Colour	... ...	Yellow.
Reaction	... ...	Neutral cold, alkaline hot.
Sperms	... ...	Lived only 1·75 minutes.

Ova fertilised and gave rise to normal embryos in fresh water in four cases only. The effect on yearling fish was very marked with this sample. As soon as the fish were introduced they showed signs of trouble at once. The jaws began to move actively, attempts at leaping out of the liquid were very frequent, then after 5 minutes a period of rest at the bottom of the vessel, the fish appearing to be exhausted, then further attempts at coming to the surface were made with gradual sinking to the bottom again. This continued for 15 minutes, when the fish showed signs of turning on their sides and within a few seconds were on their backs. Then spasmodic darts took place in an aimless manner which continued periodically for about an hour when the fish died.

Repeating this experiment but removing to fresh water at the first signs of turning over it was found possible to recover them and in a few days they acted quite normal.

Diluting this sample 1 in 4 the period of being overcome was lengthened, but the final result was the same.

Sample 6. This was from the same source as 5, but taken from the outflow pipe before it entered the stream.

Analysis of this sample showed it to be crude sewage.

Colour	... ...	Brown.
Reaction	... ...	Distinctly alkaline cold or hot.
Smell	... ...	Very offensive, ammonia, sulphuretted hydrogen prevailing.
Analysis	... ...	Parts per 100,000.
Free and saline ammonia		5–6.
Albuminoid ammonia	...	1–1·1.
Oxygen absorbed in two hours at 27° C.	...	8–9.

Sample shaken up showed froth at top which remained for 24 hours before disappearing (in a purified sample froth should disappear in three minutes). Sperms were active in this sample for one minute only. Ova fertilised in this crude sewage and removed to fresh water gave normal embryos, only four hatching out.

Yearlings placed in this sample would not live more than 5 minutes and throughout the period of emersion were in a violent state of activity.

Sample 7. This was a sample taken from the effluent pipe from a distillery when a full discharge was taking place.

A large amount of suspended colloidal matter was present which microscopic examination showed to be broken-down yeast cells and starch grains, some in an unbroken condition and others much broken up. The whole was of a whitish colour with a large amount of finely suspended matter very like starch paste.

The cloudiness took several days to disappear on standing when a mass of fungal hyphae appeared on the bottom.

Reaction ... ... Slightly acid cold; neutral hot.

Tested for presence of CO_2 showed a large amount present. Milt and ova were not available when this sample was taken and only its effects on yearling trout was ascertained.

This was peculiar, for almost as soon as introduced the fish became very sleepy, remaining at the bottom of the vessel till finally overcome within an hour and if left half an hour longer died.

Repeating this experiment but removing to fresh water at the end of an hour, when first overcome, the fish recovered its normal position within 10 minutes, indicating that crude distillery waste puts fish out of action probably through the excess of CO_2 present and the deficiency of free oxygen. This experiment has been repeated using freshly drawn distillery wash from a London distillery and the same effect obtained.

The amount of oxygen in solution rarely reached more than 2 c.c. per litre against a normal 6–7. Well aerating a sample of distillery wash improved matters especially after standing for some time to allow the colloidal matter to settle out. The addition of lime water or powdered lime helped to improve the liquor so that it was able to support fish-life if most of the CO_2 was removed and oxygen introduced.

Summary of Results.

	Sample	Colour	Reaction	Debris	Life of sperms mins.	Effect on fish	Remarks
1.	Pure water	None	Neutral	None	3	Unaffected	90 % eggs hatched out
2.	Diluted distillery effluent	None	Hot or cold neutral	None	2–3	..	1 distillery, 90 % eggs hatched out
3.	Diluted woollen mill effluent	Slightly yellow	Neutral cold, alkaline hot	Small amount	2-0	Slightly affected	80 % hatched out
4.	Diluted woollen mill effluent	Slightly yellow	Alkaline, hot & cold	Small amount	2-0	Uneasiness	80 % hatched out
5.	Sewage	Yellow	Neutral cold, alkaline hot	Small amount	1-75	Fatal	Contained large percentage sewage
6.	Crude sewage	Brown	Alkaline cold, alkaline hot	Small amount	1-0	Fatal	Very offensive, putrid smell. 40 % eggs hatched out
7.	Crude distillery waste	Whitish	Slightly acid cold, neutral hot	Small amount	—	Fatal	Contained a lot of coal dust matter. Also yeast and starch grs

Field-work was done in the area from which the samples were drawn to determine how far the laboratory experiments agreed with the conditions present.

The distillery effluent has a very marked effect on the bed of the stream.

A very small tributary of the main stream on the banks of which only one distillery is present was selected.

Above the distillery the water was clear and fish were abundant, below the distillery for about a mile no fish were found. The distillery effluent is allowed to settle a little but reaches the stream as a yeast-coloured liquid. The stream is coloured a short distance below outflow and as the debris settles a filamentous fungus appears covering the stones with a grey flocculent growth.

The species of fungus was not determined but it covers the stones for over a mile down the stream when it gradually thins out where the stream becomes normal again and fish appear. When the fungus has grounded it turns black and gives rise to a black slimy mud of a very offensive nature and makes the stream look black.

The distillery effluent contains a large amount of nitrogen from the yeast and barley and unused up starch grains. Below the woollen mill no fish were present on account of the high alkalinity which prevails at times together with waste dye-stuffs. The crude sewage gives rise to fungal growths very similar to that from the distilleries, but in less amounts.

Conclusions.

1. That distillery effluent and crude sewage is detrimental to the life of sperms and fish if poured into a stream untreated.

2. That the contents of distillery effluent give rise to fungal growths, preventing algal and flowering plants from growing and aerating the water.

3. That the plant and animal life of a stream is affected by crude trade wastes and untreated sewage entering it.

II. BIOLOGICAL PROBLEMS CONNECTED WITH A TROUT FARM

Some two years ago my attention was drawn to a serious trouble, which frequently occurs in the rearing of trout for re-stocking streams, at a trout farm in the south of Scotland; and as a result of the investigation appeared to be of economic importance, it was thought worth while to record it.

The disease is one only found in northern areas and known as "Bloom." It attacks young fry a few weeks after hatching when the food-sac is all used up and artificial feeding has begun. Often the disease continues all through the summer, only fry which are mildly attacked surviving.

The attack takes the form of a bluish appearance, arising on the flanks of the fish just behind the gill covers, gradually extending backwards towards the tail, during which time the fish get perceptably thinner and ultimately succumb a few weeks after the first attack.

The hatchery in question is served by two streams from which raceways direct the water to the various parts of the farm. Both streams drain uncultivated hillsides, and the water in both cases is the usual brown colour common to Scottish burns. After passing through the hatchery, the water returns to the main stream and flows away to the sea.

The subsoil from which the streams draw their water is of granite covered with a thick layer of peat, and the volume of water flowing through the hatchery is about 300,000 gallons per hour.

The hatchery is situated in a hollow completely surrounded with hills one of which slopes down into the grounds, and in pre-war days was covered with spruce which has since been removed. The conditions of the water draining from this area being considerably altered in consequence.

At the base of this hill a large number of sphagnum bogs occur and the water passing through these bogs, though perfectly clear, has a very detrimental effect on fish-life as repeated experiments have shown.

It does not seem to make much difference whether the experiment is tried before or after a period of drought, or in spring or summer, the result is the same, and yet not a yard separates the burn from the bog-water at some places.

Before deforestation of the hillside, the surface water was allowed to mix with that of the burn water running into the hatchery, but on account of its deadly character an extensive process of draining has been undertaken to prevent any of it reaching the water of the hatchery.

It may be a coincidence, but the altered character of the surface drainings since deforestation is very marked.

In a later paper I hope to deal more fully with this sphagnum water.

Bloom.

The symptoms of this disease have already been mentioned. It can easily be removed by putting the fry into a solution of common salt about 5 per cent. strength, but the bloom soon returns if the fish are put back into the same water from which they were drawn.

Microscopic examination of the bloom gives no clue as to its composition as it appears as a homogeneous mass of slime, no bacteria are present, nor do cultures from this slime give any positive results. Chemical investigations show it to be coagulated mucous due to the high acidity of the peat water at times, and the presence of vegetable toxins.

It has been stated that the hatchery is served by two streams, one draining a small area and the other a larger one. Taking the acidity of the two streams after a period of normal steady weather, and using phenolphthalein as indicator and boiling the samples before titrating, the acidity of the two streams calculated as sulphuric acid give:

Stream from large area, 3·62 parts acid per 100,000
Stream from small area, 1·03 ,, ,,

It was found necessary to use N 100, NaOH and 200 c.c. quantities of water to get a good end point of the acidity.

It is found that the acidity varies considerably over long periods; given a period of settled weather the acidity rarely varies from the above, but after a storm (say two or three hours) the acidity drops quickly for about an hour, then rises quickly; within an hour I have known it rise

to three or four times above the normal. The temperature may fall a degree or two but not to any marked extent.

After the acidity has risen it remains high for some hours and then very gradually comes down, taking often several days to get anywhere near the normal figure.

It is known by long experience that sudden changes in the weather are very trying for fish under artificial conditions and many young fish are lost on this account, older fish being able to withstand the changes much better than the young ones.

It has been found that the bloom makes its appearance immediately after this sudden rise in acidity, and from all experiments tried on the spot (and repeating as near as possible in glass tanks the condition of sudden rise in acidity, keeping the fish under observation all the time) it is found possible to produce artificially a somewhat similar appearance to what occurs at this hatchery, not forgetting that the water at the hatchery also contains a certain amount of vegetable matter in suspension and a certain number of vegetable toxins.

Dachnowsky in the *Botanical Gazette*, 1909, gives an account of vegetable toxins which are detrimental to animal and plant life and which occur in peat water at different times of the year.

Examination of a large number of fish which have died through bloom always shows the mouth and gill covers extended to their widest extent with the tips of the gill filaments covered with a thick layer of coagulated mucous, the stomach is invariably swollen, containing no food but varying amounts of coagulated mucous.

The first quarter of an inch of the intestine immediately following the pyloric end of the stomach is usually inflammed, due, I take it, to the high acidity of the contents of the stomach set up by the acid mucous.

Many experiments have been tried to effect a cure both on the spot and in the laboratory. Various reagents have been tried, but the most effective appears to be powdered lime, chalk, or lime water.

It has been found that causing the water to run through chalk filters, which hardens the water a little and brings down the acidity, is effectual; as is also the careful addition of lime to the water at a point where no free lime would be able to reach the hatchery. But the easiest and the most economical way has been by using lime water.

Calculating the average rate of flow and the highest acidity known to occur, it has been found possible by turning some of the ponds into lime-pits, and arranging that a certain amount of lime water flows in gradually to so regulate matters that this bloom no longer appears.

The questions of the variation and composition of the dissolved gases are now under consideration, together with the composition of the water at varying seasons.

The conclusions to be drawn from this series of observations are:

1. That deforestation considerably alters the character of the water flowing from hillsides so far as fish life is concerned.

2. That the altered water in this instance has a very detrimental effect on fish life especially in the young stages.

3. That too high acidity of the peat water may cause the coagulation of the mucous on the gills, and sides of the fish, which may be fatal.

4. That a careful control and adjustment of the acidity of the water is necessary to ensure the non-appearance of this bloom, and lime water or chalk is the most effective.

(*Received January 19th*, 1922.)

OBITUARY NOTICE

Dr CAROLINE BURLING THOMPSON.

1869–1921.

Dr Caroline Burling Thompson, Professor of Zoology at Wellesley College, Mass., U.S.A., died on December 5, 1921. Prof. Thompson was noted not only for the excellence and thoroughness of her original methods of teaching, but also for her original research work in biology. She was an inspiration to her students and also found means of helping them in many practical ways, unknown to any but herself.

Miss Thompson did original research work in biology in connection with the marine laboratories both at Naples, Italy, and Woods Hole, Mass. Her most noted work was on the biology of termites—the most destructive of the social insects. She has been a Collaborator of the Branch of Forest Entomology, Bureau of Entomology, U.S. Department of Agriculture, since March 1917.

1916 saw Miss Thompson's first paper on termites. It was an original piece of research on the brain and frontal gland of a common termite of eastern United States. She discovered that there was very little differentiation between the brains of the different castes of this termite and none between the sexes, the most marked difference being in the optic apparatus. Miss Thompson suggests that the frontal gland may have arisen phylogenetically from the ancestral median ocellus now lacking. This work was of considerable importance, since the frontal gland is of great taxonomic value.

In 1917, a paper on the origin of the castes of a common termite revolutionised the attitude taken by students of termites. Hitherto the attitude had been almost entirely anthropocentric; Dr Thompson disproved that the "complementary" or "substitute" queens or reproductive forms of termites could be manufactured through feeding by workers. She definitely proved that the origin of all castes is due to intrinsic causes. Thus, by careful scientific study, much of the mystery of the "complex" social system of the termites—which has led to admiration by man of these insects—has been proven a myth. Facts now supplant the older fantastic theories, so dear to writers of the eighteenth and nineteenth centuries!

Another paper in 1919 discussed the phylogeny of the termite castes and outlined breeding experiments which were in progress at the time of her death. It was hoped to work out a genetic formula for termites.

These papers were followed by several others on the development of the castes and reproductive forms of species of many genera of termites.

Work on the development of the castes of the honey bee had been planned and material fixed ready to section. It is to be regretted that ill-health and other duties interfered. Miss Thompson was undertaking this work as she ever did with an open mind—realising that very careful work had been done on the honey bee and that no generalisations could be made in advance. The social insects often radically differ in habits. What might be an anthropocentrism in case of the termites, might be a fact in the biology of the honey bee!

With two other co-workers, Miss Thompson was working on a more or less popular book on termites and her share was to be the internal anatomy of termites as well as phylogeny and genetic work.

A kindly, helpful spirit, of keen mind, but modest—Miss Thompson will be long remembered by her students and co-workers in science. A striking point in Dr Thompson's personality, in fact its keynote, and which signalised her as an investigator and as a teacher, is that with all her splendid training and her admirable technique she was not biased by the current fashions of the school in which she was trained but struck out into new fields. Her own research work will endure for ever!

F. E. Snyder,

Specialist in Forest Entomology, United States Department of Agriculture.

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1920 Darbishire, Prof. O. V., Ph.D., B.A., F.L.S., *Botany School, The University, Bristol.*

1920 Davey, Miss A. J., M.Sc., *Botany School, University College, Bangor.*

1915 Davidson, J., D.Sc., F.L.S., F.E.S., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Deacock, R. J., B.Sc., *School House, Crundale, Canterbury.*

1914 Deakin, R. H., *Joan Cottage, Bamford, Derbyshire.*

1920 Delf, Miss E. M., B.A., D.Sc., F.L.S., *Westfield College, Hampstead, N.W.* 3.

1915 Doidge, Miss E. M., M.A., D.Sc., F.L.S., *Division of Botany, Department of Agriculture, Pretoria, South Africa.*

1920 Dowson, W. J., M.A., F.L.S., *R.H.S. Gardens, Wisley, Ripley, Surrey.*

1920 Drummond, J. M., M.A., F.L.S., *Scottish Society for Research in Plant Breeding, Craigs House, Corstophine, Midlothian.*

1920 Duffield, C. A. W., F.E.S., *Stowting Rectory, Nr. Hythe, Kent.*

1920 Dykes, Wm. R., M.A., L.-ès.-L., *Royal Horticultural Society, Vincent Square, London, S.W.* 1.

1917 Eltringham, H., M.A., D.Sc., F.E.S., 6, *Museum Road, Oxford.*

1914 Emptage, W. F., 1, *Ullswater Road, West Norwood, S.E.* 27.

1916 Eyre, J. A. Vargas, D.Sc., *The Linen Industry Research Assoc., The Research Institute, Lambeg, Belfast.*

1920 Fahmy, T., 63, *Avenue Shoubra, Cairo.*

1909 Farmer, Prof. J. B., M.A., D.Sc., F.R.S., *Imperial College of Science, London, S.W.* 7.

1920 Fenton, E. Wyllie, *Botanical Department, Seale-Hayne Agricultural College, Newton Abbott, Devon.*

1920 Fisher, R. A., M.A., *Rothamsted Experimental Station, Harpenden, Herts.*

1905 Freeman, W. G., A.R.C.S., B.Sc., *Royal Botanic Gardens, Trinidad.*

1919 Fryer, C. H., 11, *St Mary's Road, Tonbridge, Kent.*

1913 Fryer, J. C. F., M.A., F.E.S., *Pathological Laboratory, Milton Road, Harpenden, Herts.*

1919 Fryer, P. J., F.I.C., *Ravenscar, Tonbridge, Kent.*

1918 Gahan, C. J., M.A., D.Sc., F.E.S., *Natural History Museum, London, S.W.* 7.

1914 Gamble, Prof. F. W., D.Sc., F.R.S., *The University, Edmund Street, Birmingham.*

1914 Gardiner, Prof. J. S., M.A., F.R.S., *Bredon House, Selwyn Gardens, Cambridge.*

1920 Gates, Prof. R. R., B.Sc., Ph.D., F.L.S., *King's College, Strand, London, W.C.* 2.

1922 Gilchrist, Miss G. G., B.Sc., *Botanical Department, The University, Bristol.*

1920 Gimingham, C. T., O.B.E., F.I.C., F.E.S., *The Bury, Offchurch, Leamington.*

1920 Glynne, Miss M. D., B.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Goodey, T., D.Sc., *School of Tropical Medicine, Endsleigh Gardens, Euston Road, London, W.C.* 1.

1908 Gough, G. C., B.Sc., 45, *Poplar Avenue, Edgbaston, Birmingham.*

1914 Gough, L. H., Ph.D., F.E.S., *Agricultural Department, Cairo, Egypt.*

1921 Gray, P. H. H., M.A., *Rothamsted Experimental Station, Harpenden, Herts.*

1905 Green, E. E., F.E.S., *Way's End, Camberley, Surrey.*

1909 Groom, Prof. P., M.A., D.Sc., *Imperial College of Science, South Kensington, S.W.* 7.

1921 Grubb, N. H., M.Sc., *East Malling Research Station, East Malling, Kent.*

1921 Guillibaud, W. H., B.A., *Forestry Commission,* 22, *Grosvenor Gardens, London, S.W.* 1.

1914 Güssow, H. T., F.R.M.S., 43, *Fairmount Avenue, Ottawa, Ontario, Canada.*

1920 Gwynne-Vaughan, Prof. Dame Helen, D.B.E., D.Sc., LL.D., F.L.S., *Botanical Department, Birkbeck College, Chancery Lane, E.C.*

1909 Hadwen, Seymour, D.V.Sci., *Dominion Experimental Farm, Agassiz, Canada.*

1920 Halket, Miss A. C., B.Sc., *Bedford College, Regent's Park, London, N.W.*

1914 Hamilton, Dr Lillias, *Agricultural College for Women, Studley.*

1920 Hampshire, P., F.R.M.S., *Bureau of Bio-Technology, 3 and 4, Queen's Square, Leeds.*

1921 Harland, S. C., D.Sc., *Shirley Institute, Didsbury, Manchester.*

1920 Henderson-Smith, J., M.B., Ch.B., B.A., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Hiley, W. E., M.A., *Research Institute, School of Forestry, Oxford.*

1920 Hill, A. W., Sc.D., M.A., F.R.S., *Royal Botanic Gardens, Kew, Surrey.*

1920 Hiscox, Miss E. R., B.Sc., *Research Institute in Dairying, University College, Reading.*

1920 Holden, H. S., D.Sc., F.L.S., *University College, Nottingham.*

1920 Hole, R. S., M.A., C.I.E., *c/o Messrs H. S. King and Co., 9, Pall Mall, London, S.W. 1.*

1919 Horne, A. S., D.Sc., F.L.S., F.G.S., *Botanical Department, Imperial College of Science, South Kensington, S.W. 7.*

1920 Horton, E., B.Sc., F.I.C., *S.E. Agricultural College, Wye, Kent.*

1920 Hunter, B. K., *Cauldron Barn Farm, Swanage, Dorset.*

1914 Hutchinson, H. P., *The Laurels, Kegworth, Derby.*

Orig. Imms, A. D., M.A., D.Sc., F.L.S., F.E.S., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Isaac, P. V., B.A., F.E.S., *2, Gledhow Terrace, South Kensington, London, S.W. 7.*

1918 Jackson, Miss D. J., *Swordale, Evanton, Ross-shire.*

1907 Jepson, F. P., M.A., F.E.S., *Department of Agriculture, Peradeniya, Ceylon.*

1920 Jewson, Miss S. T., B.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Jones, Prof. W. Neilson, M.A., F.L.S., *Bedford College, Regent's Park, London, N.W.*

1918 Kannan, Kunui, M.A., F.E.S., *Asst. Entomologist, Govt. of Mysore, Bangalore, S. India.*

1915 Keeble, Prof. F., C.B.E., M.A., Sc.D., F.R.S., *Botany School, Oxford.*

1920 Kidd, F., M.A., D.Sc., *Botany School, Cambridge.*

1907 King, H. H., F.L.S., F.E.S., *Gordon College, Khartoum, Egypt.*

1907 King, Prof. L. A. L., M.A., *West of Scotland Agricultural College, Blythswood Square, Glasgow.*

1921 Lacey, Miss M. S., B.Sc., *Botanical Department, Imperial College of Science, London, S.W. 7.*

1921 Lamb, S. C., *c/o Chivers and Sons, Ltd., Histon, Cambridge.*

1921 Laurie, Prof. R. D., M.A., F.Z.S., *University College of North Wales, Aberystwyth.*

1908 Lees, A. H., M.A., *National Fruit and Cider Institute, Long Ashton, Bristol.*

1909 Leiper, Prof. R. T., M.D., D.Sc., *School of Tropical Medicine, Endsleigh Gardens, Euston Road, London, W.C. 1.*

1914 Lesley, J. W., M.A., *Emmanuel College, Cambridge.*

1915 Lister, A. B., B.Sc., D.I.C., *Experiment Station, Turner's Hill, Cheshunt, Herts.*

1921 Lobjoit, William, *Ministry of Agriculture, 4, Whitehall Place, London.*

1922 Lowe, L. T., B.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*

1919 Lloyd, Llewellyn, D.Sc., *Cartref, Slingsby, Malton, Yorks.*

Orig. MacDougall, Prof. R. S., M.A., D.Sc., F.E.S., F.R.S.E., 9, *Dryden Place, Edinburgh.*

1914 McClellan, F. C., M.R.A.C., F.L.S., *Director of Agriculture, Zanzibar.*

1920 McKenzie, Miss A. D., *Ministry of Agriculture, 10, Whitehall Place, London, S.W. 1.*

1920 McLean, Prof. R. C., M.A., D.Sc., F.L.S., *Botany School, University College, Cardiff.*

1920 McLean Thompson, Prof. J., M.A., D.Sc., F.R.S.E., *Botany School, The University, Liverpool.*

1909 Mangan, Prof. J., M.A., *University College, Galway.*

1919 Mangham, Prof. S., M.A., *Botany School, University College, Southampton.*

1917 Mann, H. H., D.Sc., F.L.S., *Agricultural College, Poona, India.*

1914 Marshall, G. A. K., C.M.G., D.Sc., F.Z.S., F.E.S., *Imperial Bureau of Entomology, Natural History Museum, London, S.W. 7.*

1920 Mason, Francis Archibald, 29, *Frankland Terrace, Leopold Street, Leeds.*

1921 Mason, T. G., M.A., B.Sc., *Imperial Department of Agriculture, Barbadoes.*

1920 Mattick, A. T. R., B.Sc., *Research Institute in Dairying, University College, Reading.*

1920 Mellor, J. E. M., B.A., 51, *Onslow Square, London, S.W. 7.*

1921 Millard, W. A., B.Sc., *Department of Agriculture, The University, Leeds.*

1914 Moore, Sir F. W., M.R.I.A., V.M.H., *Royal Botanical Gardens, Glasnevin, Dublin.*

1922 Morland, M. T., M.A., *Pathological Laboratory, Milton Road, Harpenden.*

1920 Morris, H. M., M.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Mosley, F. O., F.L.S., *Laboratory of Plant Pathology, University College, Reading.*

1914 Munro, Dr J. W., *Forestry Commission, Royal Botanic Gardens, Kew, Surrey.*

1920 Murphy, A. J., 2, *Dorset Square, London, N.W. 1.*

1920 Murphy, P. A., B.A., *Seeds and Plant Disease Division, Royal College of Science, Dublin, Ireland.*

1914 Neave, S. A., M.A., D.Sc., F.Z.S., F.E.S., *Imperial Bureau of Entomology, 41, Queen's Gate, London, S.W. 7.*

Orig. Newstead, Prof. R., M.Sc., F.R.S., A.L.S., *School of Tropical Medicine, The University, Liverpool.*

1920 Nowell, W., *Department of Agriculture, Trinidad, West Indies.*

1920 Oliver, Prof. F. W., M.A., D.Sc., F.L.S., F.R.S., *University College, Gower Street, London, W.C. 1.*

1910 Osborn, Prof. T. G. B., M.A., M.Sc., *The University, Adelaide, S. Australia.*

1919 Paine, S. G., D.Sc., F.I.C., *Imperial College of Science, London, S.W. 7.*

1920 Palmer, Ray, *Ingleholme, Norton Way, S. Letchworth.*

1920 Pammel, Prof. L. H., Ph.D., *Agricultural College, Ames, Iowa, U.S.A.*

1920 Parker, T., *The Cedars Laboratories, Sheen Lane, Mortlake, London, S.W. 14.*

1914 Pearson, J., D.Sc., *Director, The Museum, Colombo, Ceylon.*

1920 Percival, Prof. J., M.A., F.L.S., *University College, Reading.*

1914 Petherbridge, F. R., M.A., *Sidney Sussex College, Cambridge.*

Orig. Pethybridge, G. H., Ph.D., B.Sc., *Royal College of Science, Dublin, Ireland.*

1914 Pole-Evans, I. B. P., C.M.G., D.Sc., M.A., F.L.S., *Division of Botany, Pretoria, Union of South Africa.*

1919 Pomeroy, A. W. J., *Govt. Entomologist, Ibadan, Southern Nigeria.*

1915 Porter, Dr Annie, *Zoological Department, S. African Institute for Medical Research, Johannesburg, Union of South Africa.*

1907 Poulton, Prof. E. B., M.A., D.Sc., LL.D., F.R.S., F.L.S., F.G.S., F.Z.S., F.E.S., *Wykeham House, Banbury Road, Oxford.*

1919 Prain, Sir David, Lt.-Col., I.M.S., C.M.G., C.I.E., M.A., M.B., F.R.S., LL.D., F.R.S.E., V.M.H., *Royal Botanic Gardens, Kew, Surrey.*

1908 Priestley, Prof. J. H., D.S.O., B.Sc., *The University, Leeds.*

1919 Rayner, Dr M. C. (Mrs Neilson Jones), *University of London Club, 21, Gower Street, W.C. 1.*

1914 Rennie, J., D.Sc., F.R.S.E., 60, *Desswood Place, Aberdeen.*

1910 Rettie, T., D.Sc., 12, *Ann Street, Edinburgh.*

1921 Richards, E. H., B.Sc., F.I.C., *Rothamsted Experimental Station, Harpenden, Herts.*

1921 Roach, W. A., B.Sc., A.R.C.S., D.I.C., A.I.C., *Rothamsted Experimental Station, Harpenden, Herts.*

1914 Roberts, A. W. Rymer, M.A., F.E.S., *School of Zoology, Cambridge.*

1916 Robinson, Wilfred, D.Sc., *Department of Botany, University of Manchester.*

1918 Robson, Robert, *Institute of Agriculture, Chelmsford.*

1920 Roebuck, A., N.D.A., *Harper Adams College, Newport, Salop.*

1907 Rogers, A. G. L., *Ministry of Agriculture, 10, Whitehall Place, London, S.W. 1.*

1920 Russell, E. J., D.Sc., F.C.S., F.R.S., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Salisbury, E. J., D.Sc., F.L.S., *Botanical Department, University College, London, W.C. 1.*

1914 Salmon, E. S., F.L.S., *S.E. Agricultural College, Wye, Kent.*

1920 Sandon, H., B.A., *Rothamsted Experimental Station, Harpenden, Herts.*

1921 Sargent, R. H., *Technical College, Darlington.*

1920 Searle, G. O., B.Sc., *Linen Industry Research Association, Glenmore Road, Lambeg, Belfast.*

Orig. Shipley, Sir A. E., G.B.E., M.A., D.Sc., F.R.S., *Christ's College, Cambridge.*

1919 Small, Prof. J., D.Sc., Ph.C., F.L.S., *Botany School, Queen's University, Belfast.*

1914 Small, W., M.B.E., M.A., B.Sc., *Department of Agriculture, Kampala, Uganda.*

1920 Smith, A. Malins, M.A., F.L.S., *Biology Department, Technical College, Bradford.*

1920 Smith, E. Holmes, B.Sc., *Botany School, The University, Manchester.*

1920 Smith, K. M., A.R.C.S., D.I.C., *The University, Manchester.*

1919 Smith, W. G., B.Sc., Ph.D., *College of Agriculture, Edinburgh.*

1913 South, F. W., *Agricultural Department, Kuala Lumpur, Federated Malay States.*

1919 Speyer, E. R., M.A., *Research and Experimental Station, Cheshunt, Herts.*

1920 Spinks, G. T., M.A., *Agricultural and Horticultural Research Station, Long Ashton, Bristol.*

1920 Stapledon, Prof. R. G., M.A., *Agricultural Buildings, Alexandra Road, Aberystwyth.*

1920 Stenhouse Williams, Prof. R., M.B., C.M., B.Sc., D.P.H., *Research Institute in Dairying, University College, Reading.*

1921 Stenton, R., *Pathological Laboratory, Ministry of Agriculture, Milton Road, Harpenden, Herts.*

1922 Stirrup, —, M.Sc., *Midland Agricultural College, Sutton Bonington, Loughborough.*

1919 Stone, H., *Forestry School, University of Cambridge.*

1919 Sutherland, G. K., M.A., D.Sc., 10, *Bank Parade, Preston.*

1921 Sutton, E. C. F., *Sidmouth Grange, Earley, Nr. Reading.*

1920 Sutton, Martin H., F.L.S., *Erlegh Park, Whiteknights, Reading.*

1905 Swanton, E. W., A.L.S., *Educational Museum, Haslemere, Surrey.*

1920 Tabor, R. J., B.Sc., F.L.S., *Botany Department, Imperial College of Science, London.*

1921 Tattersfield, F., B.Sc., F.I.C., *Rothamsted Experimental Station, Harpenden, Herts.*

1913 Taylor, F. H., *Dalmally Station, via Roma, Queensland.*

1915 Taylor, H. V., M.B.E., A.R.C.S., B.Sc., *Ministry of Agriculture*, 10, *Whitehall Place, London, S.W.* 1.

1919 Thomas, Miss E. N. Miles, D.Sc., F.L.S., 8, *Inglewood Mansions, West End Lane, London, N.W.* 6.

1921 Thornley, A., M.A., F.L.S., F.E.S., F.R.H.S., F.R.Met.Soc., *County Education Office, Shire Hall, Nottingham.*

1920 Thornton, H. G., B.A., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Tillyard, R. J., M.A., Sc.D., D.Sc., F.E.S., *Cawthron Institute, Nelson, New Zealand.*

1915 Treherne, R. C., *Department of Agriculture, Ottawa, Canada.*

1919 Trow, Principal A. H., D.Sc., F.L.S., *University College, Cardiff.*

1913 Urich, F. W., *Board of Agriculture, Port of Spain, Trinidad.*

1921 Voelcker, J. A., M.A., B.Sc., Ph.D., F.I.C., 1, *Tudor Street, London, E.C.*

1920 Wadsworth, R. V., *Cadbury Bros., Ltd., Research Laboratory, Bournville, Birmingham.*

1920 Wager, H. H., D.Sc., F.R.S., F.L.S., "*Hendre,*" *Horsforth Lane, Far Headingley, Leeds.*

1920 Wakefield, Miss E. M., M.A., F.L.S., *Royal Botanic Gardens, Kew, Surrey.*

1905 Walker, A. D., *Ulcombe Place, Nr. Maidstone, Kent.*

1914 Wallace, Prof. R., *The University, Edinburgh.*

1920 Waller, John Claude, B.A., *S.E. Agricultural College, Wye, Kent.*

Orig. Warburton, Cecil, M.A., *Yew Garth, Grantchester, Cambridge.*

1913 Wardle, R. A., M.Sc., *Zoological Department, The University, Manchester.*

1920 Ware, W. M., B.Sc., *Brookfield, Fremington, Barnstaple, N. Devon.*

1922 Warington, Miss K., B.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*

1914 Waterston, J., M.A., D.Sc., *Nat. Hist. Museum, South Kensington, S.W.* 7.

1920 Watt, A. S., B.A., *Forestry Department, The University, Aberdeen.*

1920 Weiss, Prof. F. E., D.Sc., F.R.S., F.L.S., *Botany School, The University, Manchester.*

1905 Wellcome, Henry S., *Snow Hill Buildings, E.C.* 1.

1918 West, Cyril, D.Sc., A.R.C.S., D.I.C., F.L.S., 7, *Colfe Road, Forest Hill, S.E.* 23.

1921 Whitehead, T., A.R.C.S., *University College of North Wales, Bangor.*

1912 Williams, C. B., B.A., 20, *Slatey Road, Birkenhead.*

1909 Williamson, H. C., M.A., D.Sc., *Fishery Board of Scotland, Aberdeen.*

1914 Williamson, Capt. K. B., *King's College for Women, Household Social Science Department, Campden Hill Road, London, W.* 8.

1919 Willis, J. C., M.A., Sc.D., F.R.S., F.L.S., *Beechcroft, Clarendon Road, Cambridge.*

1920 Wilson, G. Fox, *R.H.S. Gardens, Wisley, Ripley, Surrey.*

1914 Wilson, Malcolm, D.Sc., A.R.C.Sc., *Royal Botanic Garden, Edinburgh.*

1921 Wiltshire, S. P., B.A., B.Sc., *Agricultural and Horticultural Research Station, Long Ashton, Bristol.*

1921 Woodcock, G. S., *Carlton Chemical Works, Glengall Road, Millwall, London, E.* 14.

1914 Wormald, H., D.Sc., A.R.C.S., *S.E. Agricultural College, Wye, Kent.*

1920 Wortley, E. J., F.I.C., M.B.E., *Director of Agriculture, Zomba, Nyasaland.*

1916 Wright, Herbert, A.R.C.S., *Mincing Lane House, E.C.* 3.

LAWS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

1. The Association shall be named "The Association of Economic Biologists."

2. The objects of the Association shall be to promote the study and advancement of all branches of Biology with particular reference to their applied aspects.

3. The Association shall consist of Honorary and Ordinary Members.

4. Each candidate for ordinary membership shall be nominated by two members. Such nomination shall be approved by the Council and confirmed by a vote of two-thirds of the members present and voting at the next General Meeting.

Every member elected shall receive notice from the Secretaries and shall continue a member until his written resignation shall be received by the Secretaries, or until membership be forfeited under the Laws.

Ordinary Members shall pay an annual subscription of Twenty-five Shillings, due on January 1st of each year, or may compound for their subscription with a sum of Twenty-five Pounds.

All Ordinary Members on first election shall pay an entrance fee of half-a-guinea.

5. Ordinary Members shall be entitled to admission to all the meetings of the Association, to vote thereat, to present papers, to take part in discussions and to receive a copy of the Association's publications.

Each member shall be entitled to introduce personally non-members to the Association's meetings.

6. Honorary Members shall be persons, *not subjects of the British Crown*, who have contributed in an eminent degree to the advancement of the science of Applied Biology. They shall be recommended by a majority of the whole Council and elected in the same manner as Ordinary Members.

The number of Honorary Members shall not at any time exceed *twelve* and not more than *two* shall be elected in any one year.

Honorary Members shall not be liable to any payments and shall each receive a copy of the Association's publications.

Their privileges shall be the same as those of Ordinary Members, but they shall not be entitled to vote at the meetings.

7. The Council shall have power, at any of their meetings, by two-thirds of the votes of those present and voting, to terminate the membership of any member whose subscription shall be one year or more in arrears, or whose membership shall, from any other cause, be undesirable. No member whose subscription is in arrears shall be entitled to vote at a General Meeting or to receive the Association's publications, nor shall any publication be sent to a new member until his entrance fee and subscription shall have been received.

8. All meetings shall be announced by circular addressed to all Members resident in the United Kingdom. The place and time of the meetings shall be decided by the Council; ten shall be a quorum at such a meeting.

9. An Annual General Meeting shall be held and shall ordinarily be the General Meeting falling nearest to the end of the year or as the Council shall decide.

At this meeting the order of business shall be:

1. The reading of the minutes of the previous meeting.
2. The reading of a report of the Council on the work of the past year.
3. The statement of the Treasurer.
4. The election of members.
5. The election of Officers and other members of the Council.
6. Other business.

10. The business of the Association shall be conducted by a Council consisting of a President, a Treasurer, the Secretaries, the Editors and twelve Ordinary Members. Two members of Council shall be designated to act as Vice-Presidents.

11. The Council shall select to retire from office at the Annual General Meeting such number of its Ordinary Members as will cause four vacancies and no member so selected for retirement, or otherwise vacating office, shall be eligible for reappointment to the Council as an Ordinary Member until after the lapse of twelve months. A list, containing the names of all members of Council who retire, and of those other members of the Association proposed by the Council to replace them, shall be sent to all members resident in the United Kingdom at least four weeks before the date of the Annual General Meeting. Any two members shall be at liberty to transmit to one of the Secretaries not less than fourteen days before the Annual General Meeting an intimation signed by them both of their desire to add the name of a member of the Association to the list of Candidates for election to the Council.

The Secretaries shall then when necessary issue to every Member resident in the United Kingdom a completed list of the Nominations out of which the Association at the Annual General Meeting shall select the names of Members appointed to fill the vacancies on the Council. In this completed list of Nominations will be stated the names of the Members proposing Candidates other than those proposed by the Council.

At the Annual General Meeting each Member present will receive a list of the names arranged alphabetically of all the Candidates proposed, and each Member who votes shall hand in person to one of the Secretaries a copy of this list on which has been indicated the names of those Candidates whom the Member voting desires to serve on the Council in place of those vacating office.

When the ballot has been declared closed the Chairman shall appoint from among the Members present two members of the Association not being Candidates for election to serve as Scrutineers. In examining the lists so handed in the Scrutineers will set aside and take no account of any ballot-paper which supports Candidates for more than the number of vacancies, and any ballot-paper which indicates the identity of the Member voting.

The Scrutineers shall report to the Chairman of the meeting the result of their scrutiny and the Chairman before the close of the meeting shall announce the result of the ballot. In the case of an equality of votes for any Candidates, the power of selection between them shall rest with the Chairman of the meeting and shall be exercised by him before he announces the result of the ballot.

The Council shall thereupon proceed to elect from their body the officers of the Association for the ensuing year.

12. The Council may fill up any vacancy that may occur in the list of Officers and Council.

13. The Council shall meet at such times as they may determine; six members shall form a quorum.

The Council shall purchase such books, instruments, specimens, furniture and other necessaries as may be required, pass the accounts and authorise their payment, and generally manage the affairs and administer the funds of the Association.

14. The Council shall appoint a Publications Committee consisting of the Editors, the Treasurer, two Ordinary Members of the Council and two Ordinary Members of the Association.

15. At a Council Meeting, prior to the Annual General Meeting, the Council shall appoint one or more Auditors to audit the Treasurer's Accounts.

16. All properties of the Association, both present and future, shall be deemed to be vested in the Council of the Association for the time being, in conformity with the provisions of the Literary and Scientific Institutions Act, 1854.

17. No new Law shall be made nor any Law altered except on the proposition of the Council or the requisition of at least ten members addressed to the Honorary Secretaries. The new Laws or alterations of Laws shall be proposed in writing, signed by the requisitionists and delivered to one of the Honorary Secretaries a month before an Extraordinary General Meeting, which shall be called for the purpose.

Such proposed new Laws or alterations in the Laws shall be printed in the circular convening the Meeting, and sent to all members resident in the United Kingdom at least two weeks before the date of such Meeting.

No new laws, alterations or amendments shall be passed except by a two-thirds majority, when not less than fifteen members are present and voting.